Chapter 1

Introduction

Background

Muscular damage might occur because of disease, sharp or blunt trauma, ischemia, exposure to extremely hot or cold temperatures, myotoxic agents, and most commonly the muscles' own contractions (Brooks 2003). Excessive muscle forces during activities of daily living and unaccustomed exercise could result in muscle damage. The extent and severity of contraction-induced damage vary depending on the type of muscle contraction, the amount of pressure, and individual's training state. For example, unaccustomed eccentric exercise (EE), a type of muscle action in which muscle is lengthening while contracting, increases the risk of muscle damage.

A prolonged decrease in muscle glycogen concentration (O'Reilly et al. 1987), myofibrillar damage along the Z-band (Friden et al. 1984), increased blood lactate concentration (Gleeson et al. 1998), increased mithochondrial Ca^{2+} concentration (Duan et al 1990) and mitochondrial swelling (Friden et al. 1983) have been reported after eccentric exercise. Lengthening exercise have also caused perturbations in resting metabolic rate up to 48 hr post exercise (Dolezal et al. 2000).

Adequate supply of oxygen, delivered by blood flow, plays an essential role in normal functioning of all cells (Pittman 2000). Factors such as catecholamines, creatine kinase, calcium^{2+}, residual metabolites (e.g. lactic acid) and temperature might affect the pattern of oxygen consumption after exercise (Gaesser and Brooks, 1984). Increased water content of muscle and increased intramuscular pressures (Friden et al. 1983), increased mithochondrial Ca^{2+} concentration resulting in impaired respiration im (Duan et al 1990), along with some other structural and metabolic alterations after EE, could also change the pattern of local blood flow and muscle oxygenation.

The effect(s) of EE on muscle oxygenation and muscle blood flow (mBF) in humans has not been thoroughly investigated. Moreover, in the few studies that have addressed this issue, disparate results have been reported. Similarly, the possible long-term effects of concentric exercise, a type of contraction in which muscle is shortening while contracting, on local muscle oxygenation have not been comprehensively studied.

The ability to obtain reliable quantitative measurements of muscle oxygen consumption is necessary to understand the mechanisms of local muscle metabolism at rest, during and after exercise (Van Beekvelt et al. 2002). Near infrared spectroscopy (NIRS), which was first described by Jobsis (1977), has been a simple non-invasive tool to estimate the proportion of regional oxygenated and deoxygenated haemoglobin/myoglobin within skeletal muscles of healthy humans, and in those with underlying pathophysiology (Cheatle et al. 1991; McCully et al. 1994; Burnett et al. 2000; Komiyama et al. 2000; Kime et al. 2003, De Blasi et al. 2005).

The analysis of surface electromyogram (EMG) signals has also been used to detect changes in the myoelectric behaviour of a muscle both during and after damage-inducing exercise (Berry et al. 1990; Felici et al. 1997; McHugh et al. 2000). However, there has been a wide range of disagreements amongst different groups who studied the effect(s) of EE on EMG data. For instance, Komi and Viitasalo (1997) and Berry and colleagues (1990) observed some increases in EMG activity after EE, while Day et al. (1998) did not find any significant change in this parameter. Similarly, Day and colleagues (1998) and Felici et al. (1997) observed some significant decreases in mean and median frequency, while Berry and associates. (1990) did not observe any change in these EMG-measured variables after EE.

Aims and objectives

This thesis sought to investigate and monitor muscle oxygenation and blood flow before and after vigorous sessions of eccentric and concentric contractions. In addition, mechanical, biochemical and myoelectric characteristics of exercised muscles were studied to establish what relationship(s), if any, existed between these characteristics and muscle oxygenation. Several objectives were proposed, and these included:

- To investigate whether there were any changes in muscle oxygenation due to exercise-induced muscle damage,
- To determine whether myoelectric characteristics of the muscle, quantified by EMG, would change due to exercise-induced muscle damage,

- To examine whether downhill walking exercise, which involved both concentric and eccentric contractions, would affect muscle oxygenation in a similar pattern to that of pure eccentric contractions of biceps brachii, and,
- To monitor muscle oxygenation before and after maximal concentric contractions, and compare the outcomes with those of eccentric contractions, within the same muscle group.

Hypotheses

To investigate the aims of this thesis several hypotheses were proposed, including:

- Muscle oxygenation would be decreased after unaccustomed eccentric contractions.
- EMG activity would be decreased due to exercise-induced muscle damage.
- Downhill walking exercise would affect muscle oxygenation in a similar pattern to that of eccentric contractions of biceps brachii.
- Concentric contractions would not induce prolonged changes in muscle oxygenation.

Rationale

The structural alterations, increased water content, or increased intramuscular pressures observed in the muscle after exercise may change the pattern of local blood flow and increase the diffusion distance of oxygen. In animal studies, significant changes have been observed in capillary luminal shapes and area up to 3 days after EE (Kano et al. 2004). In addition, downhill running impaired muscle microcirculatory flow as well as the balance between oxygen delivery and consumption at the onset of exercise (Kano et al. 2005). An impaired oxygen transport and reduced oxygen availability could, therefore, result in some of the metabolic changes observed after EE.

The current literature has failed to acknowledge a definite pattern of change in muscle oxygenation after exercise-induced muscle damage. In fact, those who have investigated

this issue have reported dissimilar results. Therefore, further investigation was deemed to be warranted to reveal possible alterations in muscle oxygenation and blood flow, following damage-inducing exercise.

Key Terminology

Certain terms and phrases were used within this manuscript that warrant definition here.

Anaerobic glycolysis- The oxidation of glucose in the absence of oxygen, the dominant glycolytic product is lactic acid

Arterioles – small tissue arteries (10 to 100 μm diameter) from which vessel to tissue oxygen transfer begins

Chromophores – A chemical group capable of selective light absorption resulting in the coloration of certain organic compounds

Deoxy-haemoglobin – Haemoglobin without bound oxygen

Eccentric exercise (EE) - A type of contraction in which muscle is lengthening while contracting

Electromyogram (EMG) - An instrument that measures the electrical response of muscle to nerve stimulation. It can be used to evaluate muscle weakness and to determine if the weakness is related to the muscles themselves or a problem with the nerves that supply the muscles.

Fast Fourier Transform (FFT) - An algorithm and computational tool, which permits frequency spectrum signal analysis of a series of data samples

Haemoglobin – A hemoprotein composed of globin and heme that gives red blood cells their characteristic colour; primarily function is to transport oxygen from the lungs to the body tissues

Hyperaemia – increased blood flow

Hypoxia – a condition when blood oxygen saturation falls to a level that induces tissue oxygen deprivation

Ischemia – reduced oxygen levels due to poor blood supply or low blood oxygen saturation. Induced oxygen stress, usually caused by mechanical obstruction producing narrowing arteries.

Isokinetic – contraction of muscle under conditions of constant velocity

Isometric – contraction of muscle in which the muscle does not shorten but force is exerted and oxygen is consumed proportional to effort.

Muscle Oxygenation – the relative saturation of oxy-haemoglobin and oxy-myoglobin

Muscle Vascularity – the blood vessels within, and supplying the muscle, consisting of arteries-capillaries-veins, estimated as 4 to 7% of muscle mass.

Near Infrared Spectroscopy (NIRS) – NIRS technology is based on the light absorbing properties of biological tissue or more specifically, the chromophores of the blood. In skeletal muscle tissue, measures the relative proportion of oxy- to deoxy-hemoglobin.

Optode – a device used to emit light (i.e. LED's) or receive light (i.e. photodiode)

Oximeter – instrument for measuring skin blood-oxygen saturation non-invasively

Perfusion – movement of blood through a tissue's capillary bed for nutrient and gas exchange

Reactive Hyperaemia – an increase in blood flow which is the result of a short term flow restriction

Abbreviations used in this thesis

ADP	adenosine diphosphate
ANOVA	analyse of variance
ATP	adenosine triphosphate
ATT	adipose tissue thickness
BMI	body mass index
Ca^{2+}	calcium cation
CE	concentric exercise
CIR	circumference
CK	creatine kinase
cm	centimetre
CV	coefficient of variation
Cytaa3	cytochrome
DOMS	delayed onset muscle soreness
ΔdeO_2Sat	oxygen desaturation volume
EE	eccentric exercise
EMG	electromyography
EPOC	excess post-exercise oxygen consumption
FFT	fast fourier transform
FG	fast glycolytic
g	gram
Hb	haemoglobin
HbO_2	oxy-haemoglobin
HHb	deoxy-haemoglobin
hr	hour
Hz	hertz
IC30	isometric contraction at 30% of maximum voluntary contraction
IC50	isometric contraction at 50% of maximum voluntary contraction
IC80	isometric contraction at 80% of maximum voluntary contraction
IU	international units
kg	kilogram

m	metre
Max	maximum
Mb	myoglobin
mBF	muscle blood flow
MDF	median frequency
MF	mean frequency
mg	milligrams
min	minute
ml	millilitre
mm	millimetre
mmHg	millimetres of mercury
MPF	mean power frequency
mV	millivolt
MVT	maximum voluntary contraction
mVO2	muscle oxygen uptake
µM	micromoles
N	Newton ($kg \bullet m \bullet s^{-2}$)
ng	nanogram
NIRS	near infrared spectroscopy
O_2	oxygen
O_2Sat	oxygen saturation
Pi	inorganic phosphate
PCr	phosphocreatine
PVD	Peripheral vascular disease
r	correlation coefficient
RM ANOVA	repeated-measures analysis of variance
RMS	root mean square
ROM	range of motion
ΔreO_2Sat	oxygen resaturation volume
s	second
SD	standard deviation
SE	standard error

SOR	perception of muscle soreness
SPSS	Statistical Package for Social Sciences
STPD	standard temperature pressure dry
Tau-ΔdeO_2Sat	oxygen desaturation rate
Tau-ΔreO_2Sat	oxygen resaturation rate
THb	total haemoglobin
UK	United Kingdom
USA	United States of America
yr	year

The above terms and abbreviations have been defined based on the purposes of this dissertation. Notwithstanding the preceding terms and abbreviation, international standard units and abbreviations have been employed where applicable.

Conclusion

Collectively, this chapter briefly presented the aims, objectives and hypotheses of the thesis, and gave a background and rationale for the conducted experiments. A broader introduction and rationale for each experiment is presented in the related chapters. However, to provide a basic and overall knowledge surrounding muscle damage and muscle oxygenation, related literature is reviewed and presented in the following section.

Chapter 2

Review of Literature

Introduction

This chapter presents a review of literature on muscle oxygenation, muscle damage and some popular techniques for the assessment of these. First, muscle oxygenation and the pathway of oxygen to the muscle are briefly discussed. Then, muscle damage and some of the mechanisms involved in damage is outlined. Signs and symptoms of exercise induced muscle damage and some of the common methods of assessing damage are reviewed next. Finally, near infrared spectroscopy (NIRS) is described in greater detail due to its application as the main instrument in all experimental studies conducted for this dissertation.

Muscle Oxygenation

Oxygen plays a vital role in the musculoskeletal system, and muscle function can be critically affected if oxygen supply is limited. The pathway for oxygen between the environment and the muscle mitochondria has been well defined (for reviews see Weibel 1999; Hoppeler and Weibel 2000; Wagner 2000). Oxygen must first be inhaled into the alveoli (within the lungs), then pass into the blood where it binds to haemoglobin. After that cardiac activity moves oxygenated haemoglobin through the vasculature to the muscles and finally, oxygen must pass from the vasculature into the muscle cells and reach the mitochondria (Wagner 2006). It is worthwhile to note that the primary tissues and organs involved in oxygen transport are arranged in series and not in parallel (Wagner 2006). This has two important implications. First, function of the weakest step (tissue or organ) might have a strong influence on performance of the total system. Therefore, the overall throughput of the transported oxygen could not exceed that of the weakest. Second, dysfunction of any single component of the oxygen transport pathway might strongly decrease exercise capacity, but improvement of a single component would usually bring little benefit (Wagner 2006).

For the purpose of this dissertation, the pathway for oxygen from the microcirculatory red cells to the mitochondria and its possible limitation(s) is outlined below. In general, oxygen must dissociate from oxyhaemoglobin, diffuse out of red blood cell and through the plasma, and then diffuse through the capillary wall, interstitium and sarcolemma.

Finally, oxygen should move within the myocyte to the mitochondria (Wagner 2000). Limitation(s) of oxygen supply could be attributed primarily to limitation(s) in diffusional conductance for oxygen in muscle or convective problem(s) in metabolism/perfusion matching (Pittman 2000; Wagner 2000). However, it has been suggested that diffusive limitation and not convective heterogeneity is the main obstacle for oxygen supply (Wagner 2000). Amongst the factors that could affect oxygen diffusion to the myocyte, Hb importantly has contributed to overall oxygen conductance in muscle, probably via oxy-haemoglobin (HbO_2) dissociation kinetics. Capillary surface and structure were also important in oxygen conductance. In contrast, muscle fibre size did not relate to oxygen conductance, and the traditionally important role of myoglobin in oxygen conductance has been argued by several studies (this has been reviewed by Wagner; 2000).

Oxygen delivery and utilization to the muscle at rest and during exercise has been widely studied in both animals and humans (e.g., Whipp et al. 2005; Ferreira et al. 2005; Wagner 2000; Grassi 2000; Barstow 1994). Depending on the exercise intensity (moderate vs. heavy), there are two phases of rise in muscle oxygen uptake (mVO_2). After the onset of a moderate exercise (i.e. below lactic acid threshold), mVO_2 rises in an exponential fashion toward the steady-state level (primary fast component phase), which occurs within the first 20 s after the onset of exercise. However, during heavy exercise (i.e. above lactic acid threshold), mVO_2 rises more slowly towards a new steady state (Whipp and Wasserman 1972).

During steady-state, the rate of mitochondrial ATP production has been closely matched to the rate of ATP hydrolysis and expressed the existence of efficient cellular mechanisms to control mitochondrial ATP synthesis in a wide dynamic range (Tonkonogi and Sahlin 2002). Energy turnover in skeletal muscle might increase by 400 times, and muscle oxygen consumption might increase by more than 100 times during physical exercise compared to the resting condition (Tonkonogi and Sahlin 2002). Therefore, exercise could be associated with perturbations of the intracellular milieu, which may alter mitochondrial function (Tonkonogi et al. 1998). During prolonged exercise, the muscle cell is exposed to elevated Ca^{2+}, phosphate and temperature for a

long period with a potential risk of mitochondrial dysfunction (Tonkonogi et al. 1998). The increased oxygen consumption and metabolic stress during prolonged exercise may result in increased production of reactive oxygen species (ROS), which may affect mitochondrial function (Tonkonogi et al. 1998).

Oxygenation kinetics during recovery after exercise has also been investigated. Muscle oxygen uptake could remain high for a few hours after exercise depending on the exercise type, duration and intensity, as well as the individuals' training state (Gaesser and Brooks 1984). Traditionally, it was believed that lactic acid is the main determinant of excess post-exercise oxygen consumption (EPOC). The term EPOC, which was introduced by Gaesser and Brooks (1984), refers to the oxygen consumption above resting requirements after the cessation of exercise. This elevated post-exercise metabolism contributes to the energy cost of exercise and hence the thermic effect of activity (Laforgia et al. 2006). It is known now that there are other factors (than lactic acid) such as catecholamines, creatine kinase, calcium^{2+} and temperature that might affect oxygen consumption after exercise (Gaesser and Brooks 1984).

The convection and diffusion of oxygen in contracting muscle, however, has not been well understood. Theoretical calculations have predicted that the significant degree of heterogeneity in red blood cell oxygen saturation, measured in resting muscle, would be enhanced in contracting muscle, but the relationship between the heterogeneity of homodynamic variables and oxygen transport has remained poorly understood (Pittman 2000). An important hypothesis regarding the oxygen diffusion has stated that extra-cellular (outside the muscle fibre) resistance to oxygen transport was a significant component of the total transport resistance between red blood cells and the mitochondrion (Pittman 2000).

Muscle oxygenation and blood flow can be detected using different 'in-vivo' and 'in-vitro' techniques. Tonkongi and Sahlin (2002) have reviewed some of these techniques. Microscopy techniques (mostly used in animal studies) have enhanced the understanding of tissue microvascular control and oxygen transfer. However, there is still a great need to develop and apply non-invasive methods to study muscular

oxygenation in humans. Since its introduction (Jobsis 1977), near infrared spectroscopy (NIRS), has been a promising tool to investigate muscle oxygenation during rest and exercise, non-invasively. The application of NIRS in human studies will be discussed in more details, later in this chapter.

Exercise and muscle damage

In addition to the incidental muscle injuries resulting from trauma, ischemia, disease and etc., muscle damage could result from muscles own contractions (Brooks 2003), which usually appears as delayed onset muscle soreness (DOMS). Depending on individual's level of fitness and activity, most healthy adults have experienced DOMS in their life. The type of contraction exerted on a muscle, regardless of the amount and duration of force exertion, has been a key factor in causing exercise induced muscle damages. For example, eccentric exercise (EE, described latter in this chapter) generates greater tension per active muscle fibre than concentric or isometric contractions, resulting in mechanical disruption of the muscle fibre (Garrett 1990; Clarkson and Sayers 1999; Lieber 2002). In fact, performing only one eccentric contraction may result in muscle damage (Morgan and Allen 1999), while damage might not occur after sessions of repetitive concentric contractions.

Damage-inducing exercise, especially eccentric contractions, might cause many mechanical, metabolic and neurovascular alterations. Some of these changes are including prolonged decreases in muscle glycogen concentration (O'Reilly et al. 1987), changes in capillary lumina shape and area (Kano et al. 2004), impairment of muscle microcirculatory flow (Kano et al. 2005), myofibrillar damage along the Z-band (Friden et al. 1984), increased blood lactate concentration (Gleeson et al. 1998), mitochondrial swelling and increased intramuscular pressures (Friden et al. 1983). Additionally, eccentrically induced muscle damage may cause perturbations in resting metabolic rate up to 48 hr post-exercise (Dolezal et al. 2000).

Signs, symptoms and methods of assessing exercise-induced muscle damage will be discussed in more detail later in this chapter. For a better understanding of the

mechanisms behind exercise-induced muscle damage, the common types of muscular contractions and/or movements are described briefly below.

Types of contractions

The muscle can generate force in different directions and dimensions, and at various speeds, which can result in a variety of movements or stability. Therefore, muscular actions could be described in different categories. Defining muscular contractions based on the changes in muscle length is a more popular category, and it is used in this thesis. However, it is worthwhile to note that, despite the long-term application of some of the terminologies that are used in this dissertation, their application in current scientific communications has been discouraged (Faulkner 2003). Nevertheless, for the purpose of this thesis, the traditional terms are used along with a reference to the new suggested phrases.

Concentric (shortening) contractions

Concentric contraction happens when the muscle shortens as it contracts (McComes 1996; Lieber 2002). When a muscle is activated and required to lift a load which is less than the maximum tetanic tension it can generate, it begins to shorten (Lieber 2002). An example of a concentric contraction is lifting a weight during a biceps curl. In concentric contractions, the force generated by the muscle is always less than the muscle's maximum (Lieber 2002).

Eccentric (lengthening) contractions

Eccentric contraction is the opposite of concentric; the muscle lengthens as it gains tension (McComes 1996; Lieber 2002). As the load on the muscle increases, it finally reaches a point where the external force on the muscle is greater than the force that muscle can create (Lieber 2002). Therefore, even though the muscle may be fully activated, it is forced to lengthen due to the high external load (Lieber 2002). During eccentric contractions, the absolute tensions achieved are very high relative to the muscle's maximum tetanic tension generating capacity (we can set down a much heavier

object than we can lift). Additionally, the absolute tension is relatively independent of lengthening velocity. This suggests that skeletal muscles are very resistant to lengthening (Lieber 2002).

Isometric (static) contraction

In an isometric contraction, the muscle is prevented from shortening by fixing both its ends. Instead of performing external work, the muscle develops tension at its point of attachment (McComes 1996). Isometric contraction usually happens when there is a tension on the muscle but no movement is made (no change in the joint angle), causing the length of the muscle to remain the same (Lieber 2002). An unsuccessful attempt to lift a heavy weight is an example of an isometric contraction.

Theoretical mechanism(s) for exercise-induced muscle damage

Regardless of the factors involved, the manner in which the injuries are manifested appears to be the same, varying only in severity (Brooks 1996). In addition, the processes of fibre repair and regeneration appear to follow a common pathway regardless of the nature of the injurious event (Brooks 1996). The exact mechanism(s) underlying damage-inducing contractions is not yet known. Experimental data show that mechanical strain on muscle is one of the primary causes of exercise-induced muscle damage (Clarkson and Hubal 2002). A hypothesis suggested by Morgan (1990) has answered many of the questions related to eccentrically-induced muscle damage.

Morgan's theory, referred to as "popping sarcomere hypothesis", has explained the concept of sudden, non-uniform sarcomere extension of muscle during lengthening contraction (Morgan 1990). Based on this theory, Morgan and Allen (1999) have suggested that the initial changes in eccentric contractions are overstretched sarcomeres randomly distributed throughout the muscle. During or after relaxation, many of these overstretched sarcomeres will reinterdigitate spontaneously and perform their normal function. Other sarcomeres will become disrupted and, therefore, overstretched during a subsequent contraction. Finally, the resulting areas of overstretched sarcomeres can propagate, leaving larger damaged areas (Morgan and Allen 1999). There are some

other hypotheses for the mechanism(s) and processes involved in muscle damage, for which describing in details is beyond the scope of this thesis.

Regeneration process after muscle damage

Although, regeneration starts within hours after injury and develops quickly within few days, the entire process requires up to 6 months in most mammals (Lieber 2002). Carlson (1973) has described the detailed cycle of muscle regeneration after injury. Generally, after muscle injury, the damaged cellular components are digested, satellite cells proliferate to form new muscle fibre building material, and satellite cell fuse to form new myotubes and muscle fibres (Carlson 1973). This cycle is explained briefly below.

After damage, the degeneration process starts with digestion of damaged cellular components by endogenous and exogenous protease enzymes released from lysosomes and macrophages (Lieber 2002). Then, the satellite cells would proliferate. These cells arrange themselves peripherally between basal lamina and sarcolemma where primary and secondary myotubes are in process of formation. Presence of basal lamina is very important for proliferation and fusion in regeneration process, whereas sarcolemma's existence negatively affects the process (Lieber 2002). Finally, satellite cells begin to align themselves along the basal lamina and fuse into myotubes. After this, muscle fibre has to fully recover its function, which may take up to 6 months.

Possible interactions between muscle damage and oxidative metabolism

Pioneer work by Lieber and Friden (1988) demonstrated that fast glycolytic (FG) fibres have been selectively damaged by eccentric contractions (Lieber and Friden 1988). This might indicate that oxidative capacity is important in determining the extent of fibre damage that occurs immediately following EE. Based on this hypothesis, Lieber (2002) suggested two putative mechanisms for EE-induced muscle damage, although these mechanisms were not experimentally proven.

In the first mechanism, early in the exercise period (i.e. within the first 10-min) FG fibres become fatigued and because of their inability to regenerate ATP, they enter a rigor or high-stiffness state. Additional EE repetitions further stretch the stiff fibres and disrupt them mechanically. This may result in the observed cytoskeletal and myofibrillar damage.

In the second mechanism, at the initial stage of eccentric contractions, FG fibres become fatigued and based on their inability to regenerate ATP, mitochondria lose its calcium buffering capacity. Consequently, the increased intracellular calcium results in activation of the calcium-activated neutral proteases, lysosomal proteases, and other cellular processes, which are calcium-mediated.

On the other hand, muscle damage may result in oedema (Nosaka and Clarkson 1996), increased intramuscular pressure and water content of the muscle (Friden et al. 1983). These changes may increase the diffusion distance of oxygen from capillary to muscle fibre. Consequently, this may result in decreased oxygen consumption and/or increased muscle oxygen saturation. In addition, muscle damage may result in increased mithochondrial Ca^{2+} concentration (Duan et al 1990), which might consequently impair respiration and ATP production (Wrogemann and Pena 1976).

Signs and Symptoms of muscle damage

Force loss

After heavy exercise, depending on the severity, duration, and type of exercise, different amounts of force loss can occur. However, in most cases, if there is no muscle damage, maximum force recovers within a few hours after exercise. In contrast, in the event of muscle damage, the force recovery might take several days or weeks. Prolonged force loss especially after eccentric exercise is one of the most valid and reliable indirect measures of muscle damage in humans (Warren et al. 1999).

Morgan and Allen (1999) have reviewed the possible mechanisms behind exercise induced muscle damage. They divided the possible causes of muscle weakness into five categories, including:

1) Alterations in the central nervous system, motor nerve, or neuromuscular junction.
2) Unexcitable muscle cells, possibly because of gross cellular damage.
3) Failure or reduction of Ca^{2+} release.
4) Changes in the Ca^{2+} sensitivity of the systems involved in muscular contraction.
5) Disorganization of the systems involved in muscular contraction.

However, the changes in organization of the sarcomere structure and changes in excitation-contraction coupling (categories 5 and 3) appear to be the main contributors to the early reduction in tension (Morgan and Allen 1999).

Allen (2001) has also reviewed and studied the mechanisms of the force reduction arising from muscle damage and suggested the following four possible mechanisms contributing to force loss:

1) A shift in the peak of the force-length curve to longer lengths resulting from weakened or overstretched sarcomeres. This would result in a decrease in isometric force at this length, if it is measured at the original length giving maximal force.
2) Alterations in excitation-contraction coupling in muscles that result in reduced Ca^{2+} release and accordingly reduced force.
3) There are degenerating and consequently unexcitable muscle fibres in many muscles.
4) Some sarcomeres are still close to their normal length but give less force, for example, because their thick and thin filaments do not reinterdigitate.

Strenuous eccentric exercise can often cause up to 50–65% force loss when compared with pre-exercise values(Clarkson and Hubal 2002). This is significantly higher than the strength loss associated with concentric exercise, which usually is 10–30% immediately after exercise. Force loss generally starts very soon after EE, reaches the lowest value in the same day or the day after, remains at the lowest amount for approximately 2 to 3 days, and then recovers slowly.

Sayers and colleagues (2000) studied the effect of immobilization and light exercise following the damage-inducing exercise and found that both treatments improved recovery. Therefore, different mechanisms could be involved in the recovery of maximum force (Sayers et al. 2000). Force recovery has been observed to follow a similar pattern in some previous studies. For instance, the recovery of baseline maximum force was 82% after 12 days in Sayers and co-workers (2000), 76% after 10 days in Nosaka and Clarkson (1994) and 78% after 11 days in Chleboun and associates (1998).

Muscle soreness

Soreness is a common finding associated with muscle damage. In most of the studies investigating exercise-induced muscle damage, a perception based scale of soreness has been employed. Soreness generally starts and increases within 24 hr, peaks between 24 to 48 hr and decreases within 5 to 7 days after eccentric exercise (Clarkson and Hubal 2002). The degree of soreness may depend on the type of exercise and on individual's fitness. Different scales have employed to assess muscle soreness, but in general, scales start from a point equal to 'no pain' and end at a point equal to 'severe pain' and soreness.

Soreness could be due to swelling and pressure in the muscle, although magnetic resonance imaging (MRI) observations indicated that oedema has not coincided with soreness (Rodenburg et al. 1994). The inflammatory responses after high force EE resulted in the accumulation of neutrophils and macrophages at the site of tissue injury (Smith 1991). Macrophages synthesize an abundance of prostaglandins, which sensitize the Type III and Type IV pain afferents, resulting in muscle soreness (Sayers et al. 2000). These substances may accumulate in the muscle during immobilization, due to a reduction in blood supply to the muscle (Sayers et al. 2000). Impaired circulation could trap noxious stimuli in the muscle, leading to an enhanced sensation of muscle soreness (Sayers et al. 2000). Proske and Allen (2005) have suggested that soreness could be an allodynia, in which changes in processing at the level of the spinal cord allow mechanoreceptors, served by large-diameter afferents, to access the pain pathway.

Plasma creatine kinase (CK) activity

Compared to other muscle proteins appearing in blood that have been assessed as indirect measures of muscle damage, plasma CK activity has gained the most attention probably because the magnitude of increase is greater than other proteins and the cost of the assay is less (Clarkson and Hubal 2002). Muscle enzymes (e.g. lactate dehydrogenase, aspartate aminotransferase, carbonic anhydrase isoenzyme II), and muscle proteins (e.g. myoglobin, heart fatty acid binding protein, troponin, and myosin heavy chain) usually change due to damage-inducing exercise. After high-force eccentric exercise (e.g. maximal contractions of the elbow flexors), CK increases from about 48 hr post-exercise, with peak activity (generally 2,000-10,000 IU) occurring about 4 to 6 days post-exercise, while after downhill running CK peaks 12-24 hr after exercise with a lesser range of increase (100-600 IU; Clarkson et al. 1992). Even in similar types of exercise, there have been large variations in the magnitude of CK increase within an individual. For example, in a study by Newham and colleagues (1983) a CK ranging from 500 to 34500 IU was observed after exercise. The type of recovery after exercise may also attribute to the CK response. Sayers et al. (2000) found that immobilization of exercise-damaged muscle during recovery significantly blunts serum CK activity, which could be due to an attenuated removal of CK from the muscle and/or a decrline in lymphatic transport.

However, any speculation based on CK and any other muscle proteins should be considered with caution, because, firstly, a muscle fibre's permeability to intramuscular enzymes might or might not be correlated with cellular contractile function (Lieber 2002). Secondly, blood concentration is a function of what is being both produced in the muscle and cleared from the blood (Clarkson and Hubal 2002). This may suggest that the ability and efficacy of other tissues and organs (e.g. cardiovascular system) could also affect the amount of CK in blood.

Swelling and oedema

Inflammation, swelling and/or oedema are common symptoms of many disease and disorders. These symptoms also emerge after muscle damage. Accumulation of fluid in the damaged area result in swelling, and when fluid accumulation exceeds capability of lymphatic drainage, oedema is produced (Guyton 1986). Swelling has been detected for several days after a single session of eccentric exercise (Nosaka and Clarkson 1996; Nosaka et al. 2002).

The increases in circumference, muscle thickness, and muscle area seem to be related to inflammatory swelling (Nosaka and Clarkson 1996; Sayers et al. 2000). Therefore, in many studies assessing exercise-induced muscle damage, limb circumference is being measured as an extra means to assess the damage, if any.

Decreased range of motion

ROM is defined as the arc over which a joint may operate, and this constrains the muscle length range. In addition to the muscle characteristics, ROM is determined by skin, subcutaneous tissue, tendon, articular capsule and bone properties (Warren et al. 1999). ROM is a reliable means of quantifying the functional decrements resulting from the injury (Warren et al. 1999). ROM provides information about both muscle shortening and lengthening ability.

Muscle shortening ability is assessed by the flexed limb angle. For elbow flexors, this angle could be assessed when subject attempts to fully flex the forearm. The ability to flex the forearm is usually decreased after damage inducing exercise (Clarkson et al. 1992; Nosaka et al. 2002). The pattern of decrease in ROM is similar to that of muscle maximal force, and the changes in both MVT and ROM are compatible with overstretched sarcomere theory (Clarkson et al. 1992). This theory explains that the eccentric contraction could stretch sarcomeres so that they could not form the maximal number of cross bridges and hence reduce strength. Similarly, the stretched sarcomeres would not be able to produce maximal sliding together of the actin/myosin filaments,

and this could affect the ability of the muscle to become fully flexed (Clarkson et al. 1992).

Relaxed arm angle can be used for the assessment of spontaneous muscle shortening. The ability to extend the forearm is usually decreased after muscle damage, and this could be due to changes in property of connective tissue or changes in tendon at its attachment (Howell et al. 1985; Newham 1988; Clarkson et al. 1992). Howell and colleagues (1985) used electromyography (EMG) to assess the myoelectrical activity of elbow flexors in eccentrically exercised muscles and observed that EMG activity did not increase as the sore arms extended passively. This indicated that extra resistance to extension associate with muscle soreness was not a result of stretch receptor-induced activity.

Degradation of Cytoskeletal Proteins

The proteins associated with the sarcomere can be categorized into three groups: (1) contractile proteins such as actin and myosin, (2) regulatory contractile proteins such as troponin and tropomyosin, and (3) structural and costameric proteins (Caiozzo and Rourke 2006).

Costameric proteins, which are also named cytoskeletal proteins, stabilizes the contractile proteins and allow for the transmission of tension both longitudinally and laterally (Morgan and Allen 1999). For instance, Titin, which is a very large-molecular-weight protein, connects the Z line to the myosin filaments. This protein is responsible for much of the resting tension in highly stretched fibres and has an important role in locating the thick filaments in the centre of the sarcomere. Titin has an important role in reinterdigitation of the sarcomere after stretch, and the gradual failure of reinterdigitation resulting from repeated eccentric stretches, could be because of stretch-induced damage in Titin (Morgan and Allen 1999).

Desmin is another structural protein mainly located in the Z disks and connecting adjacent Z disks and Z disks at the edge of the fibre to the costamere in the surface

membrane (Morgan and Allen 1999). Therefore, desmin plays a key role in aligning the Z-line of adjacent sarcomeres (Caiozzo and Rourke 2006). In addition, desmin is involved in lateral transmission. Hence, desmin loss that might occur due to the activation of proteases, could be involved in the cell damage (Morgan and Allen 1999).

The site of connectivity between the sarcomere-cell membrane-extracellular matrix has been named *costameres*, and there is a growing list of proteins such as dystrophin and the integrins associated with these complex (Caiozzo and Rourke 2006). Dystrophin involves in the formation of a glycoprotein complex located in the sarcolemma but with attachment to actin filaments (Morgan and Allen 1999). Loss or dysfunction of dystrophin or other proteins in this complex leads to muscular dystrophy in which damage to fibres is a prominent feature (Morgan and Allen 1999).

Methods of evaluating muscle damage

Biopsy

Muscle biopsy is a general term referring to the removal for diagnostic study of a piece of tissue from a living body. Friden and co-workers (1981) were amongst the first investigators who applied muscle biopsies in their investigations. They observed that two and seven days after a session of stair descents, muscle biopsies of the soleus muscle showed myofibrillar disturbances and Z-line streaming. Newham and colleagues (1983) observed a greater damage in the biopsy samples 24 to 48 hr taken after exercise compared to those taken immediately after exercise.

Despite the popularity of biopsy sampling in exercise and sport science, it is not a recommended method of investigating the extent of muscle damage because of the following concerns. First, taking biopsy is an invasive procedure. Second, due to the fact that only a small portion of the muscle is sampled, the results may not be representative of the true muscle damage. Third, some studies have shown that biopsy itself may cause muscle damage (e.g., Malm et al. 2000; Roth et al. 2000). Taking multiple biopsies over a period of 7 days, resulted in similar changes in infiltrating

neutrophils and macrophages in both control and exercise (eccentric cycling) groups (Malm et al. 2000).

Magnetic Resonance Imaging (MRI)

MRI technique has been used in muscle damage studies since 1991. Although MRI is expensive, it is a precise and reliable technique to investigate damaged tissues. The T2 (transverse or spin-spin) relaxation time of muscle in proton magnetic resonance images increases during exercise and returns to the resting values within 1 hr post exercise (Ploutz-Snyder et al. 1997). This acute change seemed to be a result of changes in intracellular water chemistry. However, there is another phase of prolonged increase of T2 relaxation time, which generally occurs after damage inducing exercises such as eccentric exercise (Shellock et al. 1991; Foley et al. 1999). Shellock and colleagues (1991) were amongst the first to observe that increased T2 develops gradually from 1 to 6 days after eccentric but not concentric or isometric exercise.

Foley and associates (1999) attempted to clarify the long-term pattern of T2 relaxation times and muscle volume changes in human skeletal muscle after intense eccentric exercise and to determine whether the T2 response exhibits an adaptation to repeated bouts. They studied T2-weighted axial fast spin echo MRI of the upper arm obtained from six adult men who performed two bouts of eccentric biceps curls, 8 weeks apart. The images were taken immediately before and after each bout, at 1, 2, 4, 7,14, 21 and 56 days after bout 1; and at 2, 4, 7 and 14 days after bout 2. Their results showed that resting muscle T2 increased immediately after both bouts of exercise. T2 peaked 7 days after bout 1 and remained elevated at 56 days. After the second bout, T2 peaked lower and earlier (2-4 days), which suggested an adaptation of the T2 response. Foley and colleagues (1999) also quantified the arm and internal muscle compartment via MRI and their results confirmed the results from previous non-MRI studies, which showed arm circumferences increased immediately after exercise and rose to a maximum that occurs 1–2 days after serum CK levels peaked (Clarkson et al. 1992; Rodenburg et al. 1994). Foley et al. (1999) also showed a 7-10 percent reduction in size of the exercised muscles that was maintained from 2 to 8 weeks after the initial exercise bout. They

interpreted this outcome as evidence for the loss of a subpopulation of mechanically weak fibres, an adaptation consistent with the enduring protection against further damage.

Ploutz-Snyder and associates (1997) applied a negative leg pressure device to create oedema by increasing extra cellular fluid volume and compared this with exercise-induced changes. Their aim was to find whether changes in muscle T2 relaxation time were due to increased intracellular or extra cellular fluid volume. They found that T2 components differed between the negative leg pressures compared to the exercise condition. Therefore, they concluded that the T2 change during fatiguing (non-damaging) concentric exercise is most likely from changes inside the muscle cells.

Ultrasonography

There is convincing evidence that muscle ultrasonography can demonstrate the muscle oedema and changes in muscle belly thickness resulting from damage-inducing exercise (Nosaka and Clarkson 1996; Chleboun et al. 1998; Sbriccoli et al. 2001). Ultrasonography is also effective in the follow up monitoring of muscle recovery after damage (Nosaka and Clarkson 1996).

Sbriccoli and colleagues (2001) used ultrasonography to examine muscle oedema and changes in muscle belly thickness over a period of 28 days after EE of forearm muscles. They found that the increase in muscle belly thickness (oedema and swelling) could be detected as early as 3 hr after EE. The maximum increase was observed between 2 to 4 days after eccentric exercise in all subjects, which was also coincided with the highest CK activity (Sbriccoli et al. 2001). Nosaka and co-workers (1996) and Chleboun et al. (1998) reported similar results.

Ultrasonography can also be used to detect changes in muscle blood flow in injured muscle (Sbriccoli et al. 2001). In addition to the standard ultrasonography, Sbriccoli and co-workers (2001) used Doppler ultrasound in order to evaluate local muscle blood flow. They observed a sudden modification in local blood flow 3 hr after EE. The

increase in echo-Doppler data accompanied by the decrease in pulsatility index indicates a reduction in the peripheral resistance with an increase in the mean velocity of the blood flowing in the muscle (Sbriccoli et al. 2001).

Although ultrasound has been widely used in medical diagnoses, its application in research is not as popular. The difficulty in documenting the images in a standardized manner, and therefore, in gaining reproducible diagnoses is the main disadvantage of ultrasound (Pate 2003).

Electromyography (EMG)

EMG has also been used to investigate muscle damage. EMG is an electrophysiological technique for quantifying motor activity in specific muscle groups as determined by electrode placement. The analysis of the EMG could be used to detect the possible changes in the electric behaviour of a muscle, associated with exercise (Felici et al. 1997; Sbriccoli et al. 2001).

Different linear and non-linear EMG parameters have been used to evaluate muscle damage and recovery after EE. Amongst them, Median frequency (MDF), root mean square (RMS), and mean frequency have been used frequently in previous studies (Merletti et al. 1995; Felici et al. 1997; Alfonsi et al. 1999; McHugh et al. 2001). RMS and MDF provide information related to:

- the number and location of the active motor units,
- the recruitment of motor units,
- the shape of the motor unit action potentials,
- the mean firing rate of the individual motor units, and
- the extent of superposition of action potentials from concurrently active motor units (Solomonow et al. 1990).

Using electrical stimulation instead of voluntary contraction, Melerti and associates (1995) observed that the initial values and fatigue indices based on amplitude variables are less repeatable than those based on spectral variables. They also reported that the M-

wave shape, rather than amplitude or width, seems to be a characteristic of individual muscles and electrode location is a critical issue in the study of M-waves elicited by stimulation of a muscle motor point. In their study, MDF and mean frequency were the most consistent and repeatable variables both in terms of initial value and fatigue indices. Apparently, MDF and mean frequency have equivalent repeatability while MDF has a slightly greater sensitivity to fatigue than mean frequency (Merletti et al. 1995).

Felici et al. (1997) investigated the possibility that the EMG from exercised muscle would show marked changes to demonstrate muscle damage after EE. Their results suggested that spectral parameters (e.g. MDF) were less sensitive to error introduced by electrode repositioning than time domain parameters (e.g. RMS), and were more sensitive to EE-induced EMG changes. Their findings also revealed that MDF followed the evolution of muscle damage. Some other studies have evidenced a decline in MDF following EE (Linnamo et al. 2000; Sbriccoli et al. 2001).

Decreased MDF after EE may be an indicative of selective damage of the fast twitch fibres in this type of exercise (Linnamo et al. 2000). Sbriccoli and colleagues (2001) studied the myoelectrical changes that occurred after a session of EE over a period of 4 weeks. They observed a significant decrease in the initial frequency content, and in the MDF decay. The EMG amplitude (RMS) was unchanged after EE. Although reduced fast-twitch fibre recruitment could be responsible for both the MVT and MDF reductions, the unmodified post-exercise RMS might indicate that recruitment after EE was unchanged (Sbriccoli et al. 2001).

In contradiction to the above studies, Kroon and Maeije (1991) observed an increase in the mean power frequency decay after EE. Although this divergence could be related to the lower effort required by subjects who participated in Kroon and Maeije's study (Sbriccoli et al. 2001). Other factors such as different methods of data acquisition and analysis could also have contributed. Similarly, some other groups have either not found any changes in EMG signals or observed different findings (Berry et al. 1990; McHugh et al. 2000). Felici and colleagues (1997) have discussed some of the reasons for this

controversy. For instance, different methods have been applied to produce muscle damage; therefore, extent and severity of muscle damage could be different and consequently dissimilar changes in EMG could be expected. Additionally, different methods have been used to quantify EMG activity. For example, the time epochs and the parameters that were used for EMG analysis in the studies by McHugh et al. (2000) and Berry and colleagues (1990) were different from what was used in Felici et al. (1997) and Sbriccoli et al. (2001). McHugh and colleagues (2000) obtained their MDF data from the MVT tests with time epochs of almost 4 s, while Felici and associates (1997) measured MDF from 50% and 80% of MVT with time epochs of 1s. In a voluntary situation such as MVT, there might be some variations in the force production due to, for instance, motivation (Linnamo et al. 2000). On the other hand, in an isometric situation at a given intensity the EMG power spectrum has been rather more reliable even for measurements repeated over separate days (Linnamo et al. 2000).

Application of a vast variety of EMG acquisition and analysis methods in human studies has made it difficult to interpret and compare the findings of different studies. Therefore, the international society of electromyography has suggested a standard format for reporting EMG data (Merletti 1999) and many researchers are currently applying this format.

Near Infrared Spectroscopy (NIRS)

The NIRS technique is a non-invasive, non-ionizing, real-time monitoring continuous and direct method to determine oxygenation and hemodynamics in tissue. It has enabled the study of local differences in muscle oxygen consumption and delivery. The method has proved to be a valid non-invasive monitor, useful for investigating the physiology of oxygen transport to tissue(Van Beekvelt et al. 2001; Boushel et al. 2001; Quaresima and Ferrari 2002). NIRS is sensitive to changes in tissue oxygenation both at the level of the small blood vessels and capillaries and at the intracellular sites of oxygen uptake (Hampson and Piantadosi 1988). Additionally, NIRS is a sensitive tool to discriminate normal and pathological states (Van Beekvelt et al. 2001).

The NIRS technology, which was first described by Jobsis (1977), is based on the light absorbing properties of biological tissue, or more specifically, the chromophores of the blood, which include haemoglobin (Hb), Myoglobin (Mb) and cytochrome. However, the NIRS signal in human tissue is derived predominantly from the absorption of light by Hb in small arterioles, capillaries and venules (Boushel et al. 2001). The theory behind NIRS technology has been well explained by many authors (e.g., Villringer and Chance 1997; Boushel et al. 2001; Quaresima and Ferrari 2002). NIRS was used in all of the experiments presented in this thesis and is the principal focus of the thesis, therefore, NIRS technique and its application is discussed in more details below.

NIRS technology

As an electromagnetic wave, visible light's radiation is in the wavelength range of 380-800 nm. The range below visible light spectrum is near ultra-violet (300-380 nm) and above is near infrared (700-1000 nm) range. At wave lengths of 700-900 nm there is a good penetration of various biological tissues of the body, which are relatively transparent at these wavelengths (Villringer and Chance 1997; Boushel et al. 2001; Quaresima and Ferrari 2002). In human studies, the NIRS technique targets oxygenated and deoxygenated haemoglobin and myoglobin, which are the primary absorbing compounds of the near infrared (NIR) wavelengths. However, for NIR light to reach the target tissue (in this case the muscle), it has to penetrate a number of different tissues such as the skin and adipose tissue.

Once NIR light has reached the designated penetration depth, it interacts with the muscle, or more importantly, the Hb and Mb within the muscle. At 750 nm, deoxygenated haemoglobin (HHb) has a much higher light absorbance while at 850 nm oxygenated haemoglobin (HbO_2) has the higher absorbency (Macdonald et al. 1999; Hamaoka et al. 2000). Based on different absorption properties of the tissue, a NIRS instrument calculates the amount of HHb and HbO_2. Figure 1 shows a NIRS probe emitting in and receiving light from a tissue.

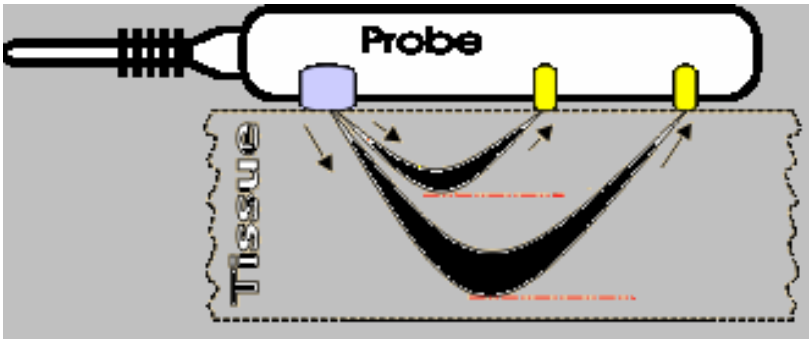

Figure 1. A NIRS probe and the pathway of NIRS wavelengths in and from the tissue.

NIRS techniques

Based on the type of light source adopted and the modality of the light sources, several NIRS techniques have been developed to investigate tissue oxygenation. Amongst these NIRS systems, there are three fundamentally different types of NIRS instruments including continuous intensity, time-resolved and intensity-modulated systems.

Continuous-intensity or continuous wave instruments which have been widely used, constantly measure the amount of light leaving the tissue surface whilst under constant illumination (Hamaoka et al. 2000; Sako et al. 2001; Kell et al. 2004). However, these instruments do not always allow quantitative measures of absolute concentrations of the chromophores, but instead provide concentration changes deviating from a baseline value (i.e. rest) during variations in oxygen availability and utilization (Quaresima et al. 2004). This limitation is due to the necessity of obtaining an accurate pathlength for each wavelength through a given tissue, and an estimate of the amount of light scattering by the tissue (Quaresima et al. 2004).

The time-resolved spectroscopy (TRS) uses photon pulses with widths of the order of picoseconds or shorter. TRS measures the temporal response of tissue to a short burst of NIR light. As shown in Figures 2 and 3, the family of photon paths produced by scattering, leads to a broadening of the pulse and depict the temporal point spread function (Arridge et al. 1992; Rolfe 2000). The light emerging from the tissue is generally recorded with highly sensitive equipment such as a synchronoscan streak camera or time-correlated single photon counting system (Hamaoka et al. 2000;

Boushel et al. 2001; Ntziachristos and Chance 2001). Although TRS has been considered the ‘‘gold standard’’ compared to continuous wave and intensity modulated systems because of providing a higher information content per source–detector pair, it requires highly sophisticated and expensive equipments (Ntziachristos and Chance 2001).

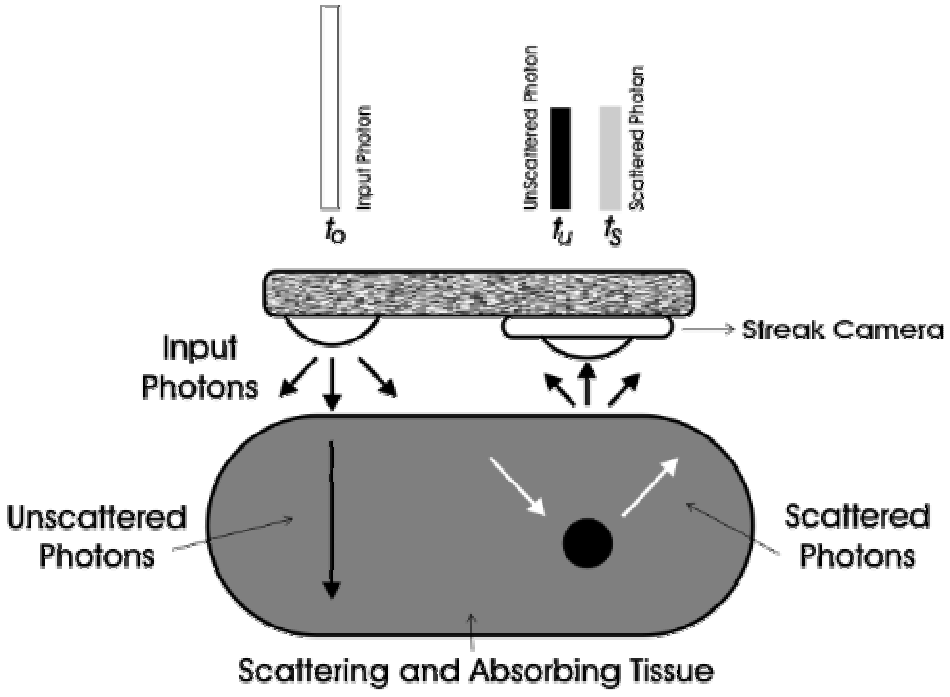

Figure 2. Depiction of the influences of scatter within a heterogeneous structure. A short pulse of light will be detected at a time dependent upon scattering events**.**

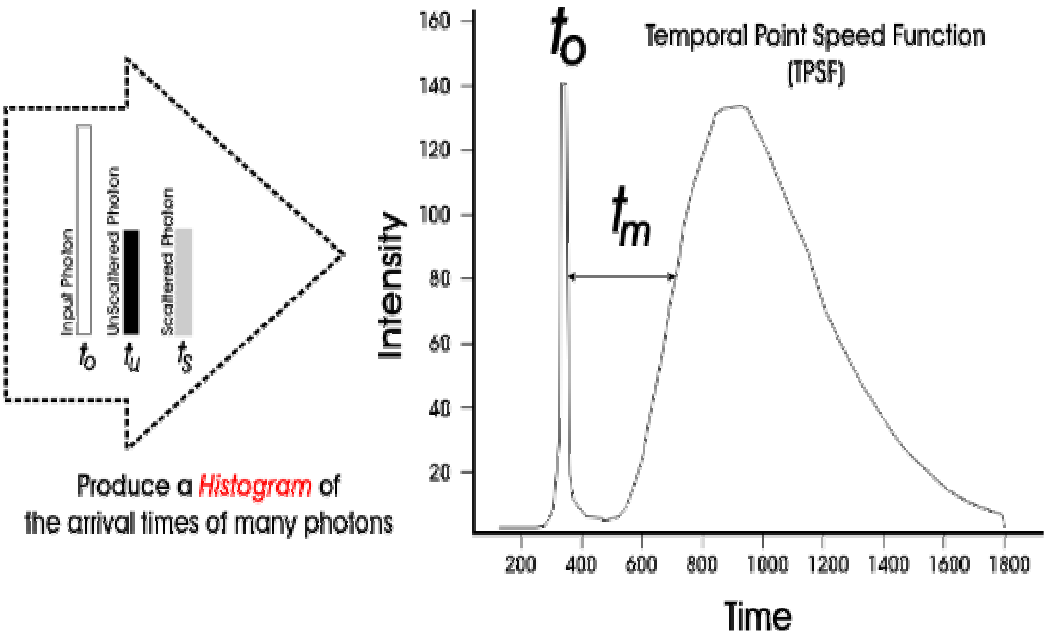

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Figure 3. The formation of the temporal point spread function (redrawn from Rolfe, 2000).

In intensity-modulated (Frequency-domain) systems, the intensity of a light source is modulated at various radio frequencies (Villringer and Chance 1997; Ntziachristos and Chance 2001). In this system, the intensity of the excitation light is modulated sinusoidally and the phase shift of the fluorescence, relative to the excitation, is determined (Figure 4). Frequency domain near infrared spectroscopy, employs an array of light sources arranged at multiple distances and frequency modulation of the near infrared light to measure light scattering and absorption (Fishkin et al. 1994; Fantini et al. 1995; Fantini et al. 1999). This technique provides the absolute measurement of the tissue absorption and reduced scattering coefficients, as well as the tissue haemoglobin concentration and saturation (Fantini et al. 1999). A frequency domain NIRS system (OxiplexTS, ISS, USA) was employed for the experiments presented in this thesis. This allowed the absolute measurement of oxy- and deoxy-haemoglobin, total haemoglobin and tissue oxygen saturation.

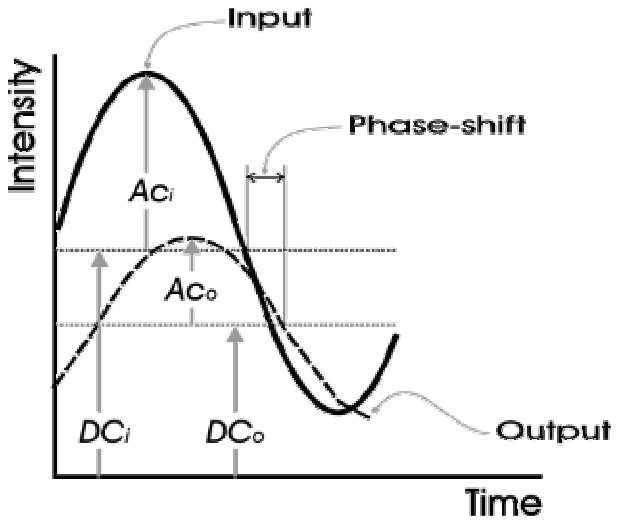

Figure 4. Phase shift and waveform parameters used in frequency domain or intensity modulated NIRS (redrawn from Rolfe, 2000).

Some technical considerations

Although NIRS technology has made reliable and valid measurements of tissue oxygenation, the method has some technical limitations that need careful consideration. NIRS function is well optimized when the NIR light passes through smaller blood vessels such as arterioles, capillaries and venules. Therefore, the type of tissue being tested is of great importance. According to the Beer-Lambert law, photons will successfully traverse through tissue with the least absorbance (Jobsis 1977), therefore, when small blood vessels are penetrated, a large number of photons will successfully contact the receiving NIRS optode providing accurate readings of changes in chromophore concentration. When light is emitted into larger vessels, there could be complete absorbance due to the large molar quantity of the blood.

Further improvement in the accuracy of NIRS signals could be obtained by considering the penetration depth. Penetration depth is approximately half of the optode separation (Hamaoka et al. 1996; Boushel et al. 2001). Therefore, specific care must be taken when choosing the separation distance between the light source and the detector. These physical properties of the probes have a high impact on the performance of the device.

Finally, although NIRS is a reliable method of monitoring tissue hemodynamics, the type and specifications of the instrument being used, should be expressed along with presented results. Comparing two different NIRS instruments, Komiyama and colleagues (2001) found a significant correlation between the muscle oxygen saturation values obtained at rest using the two oximeters, whereas these values were significantly different during arterial occlusions. Therefore, it was suggested that the operating range of the tissue oximeters should be recognized and indicated (Komiyama et al. 2001).

NIRS technical limitations

Skin is the first barrier that must be traversed by NIR light. It is one of the most highly scattering mediums within the body; however, with adequate optode separation, this scattering is attributed to less than 5% of the NIRS signal (Hampson and Piantadosi 1988; Mancini et al. 1994; Boushel et al. 2001). Mancini and associates (1994) studied the effects of various skin flows, using laser flow Doppler and NIR recordings during hot water immersion. Doppler measurements recorded significant variations in skin flow, while the NIR signal was minimally affected.

Adipose tissue also may affect the accuracy of NIRS signals. An individual with high levels of subcutaneous tissue would have a reduced muscle penetration or NIR light due to the increased depth of the skeletal muscle from the light source (Boushel et al. 2001; Van Beekvelt et al. 2001). In the case of a subject with high subcutaneous fat deposition, there would be a reduction in signal accuracy. This could be due to blunting of the received NIRS signal because of the lower metabolic and blood flow rates in adipose tissue (McCully and Hamaoka 2000). Therefore, large adipose tissue thickness may have a substantial confounding influence on NIRS measurements (Van Beekvelt et al. 2001; Wolf et al. 2003). Although recent findings of Maikala and Bhambhani (2006) did not support this, NIRS signal comparisons between individuals of varying body fat should be treated cautiously.

Variables that can be measured by NIRS

Some of the variables that could be directly or indirectly measured by NIRS include THb, HbO_2, HHb and tissue oxygen saturation (O_2Sat), oxygen index, mVO_2, mBF, recovery time and the vascular compliance in skeletal muscle. mVO_2, mBF (De Blasi et al. 1993), the alterations in Hb, HbO_2, changes in tissue and venous oxygen saturation, and oxygen index (Quaresima et al. 2003) can be measured during exercise.

After instrument set up and calibration, in most human studies a preliminary venous and/or arterial occlusion had been applied to normalize the raw NIRS-measured data and to estimate the resting mBF and mVO_2. For instance, one could assume the resting oxygen saturation obtained prior to a super systolic arterial occlusion as the 100% O_2Sat and the nadir reached during occlusion as 0% O_2Sat. Accordingly, the consequent changes induced by the investigated event (e.g. exercise) could be presented as a percentage of the changes from rest (100%) to nadir (0%). Figure 6 demonstrates a sample of NIRS-measured tissue oxygen saturation obtained during venous and arterial occlusions, as well as isometric contractions at 50% and 80% of maximum voluntary torque.

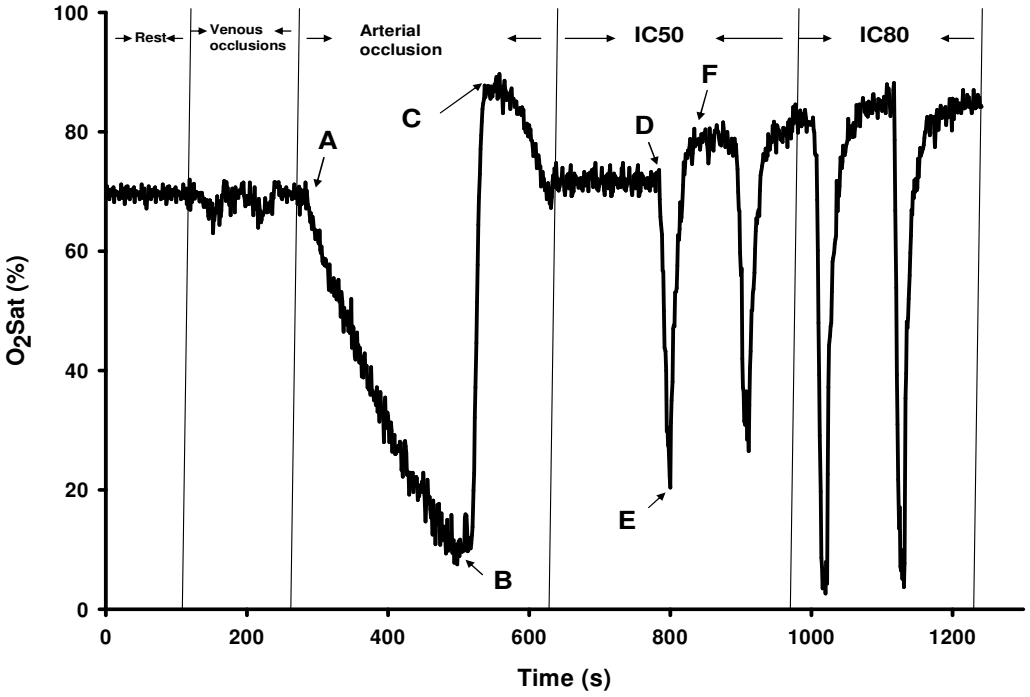

Figure 5. A sample of NIRS-measured tissue oxygen saturation (O_2Sat) obtained during upper arm venous and arterial occlusions, as well as isometric contractions at 50% (IC50) and 80% (IC80) of maximum voluntary torque. Key: A- resting O_2Sat at the beginning of arterial occlusion. B- The minimum O_2Sat achieved during arterial occlusion. C- The maximum O_2Sat achieved after releasing arterial occlusion. D, E and F- Resting, nadir and maximum O_2Sat, respectively, obtained during IC50.

Validity, sensitivity and reliability of NIRS in exercise-related studies

Validity of the quantitative measurement of muscle oxidative metabolism during exercise using NIRS had been studied (Sako et al. 2001) and a significant correlation has been observed between muscle oxidative metabolic rate (measured by NIRS) and creatine phosphate resynthesis rate (measured by magnetic resonance spectroscopy, MRS) 30 s after exercise. The NIRS feasibility and methodology to observe the deoxy Mb and Hb signals in skeletal muscle has also been established (Tran et al. 1999). Mancini and colleagues (1994) suggested that 760-800 nm absorption was closely correlated with venous oxygen saturation, minimally affected by skin blood flow, altered by changes in limb perfusion, and primarily derived from HHb and not Mb.

NIRS can be applied as a powerful tool for the investigation of spatial and temporal features of changes in muscle oxygenation (Quaresima et al. 2001; Torricelli et al. 2004; Quaresima et al. 2004). Kell and associates (2004) described the trends in oxygenation and blood volume of the right and left paraspinal muscles during the Biering-Sorensen muscle endurance test using NIRS, and assessed the test-retest reliability of oxygenation and blood volume changes during this test in healthy males. They suggested that oxygenation and blood volume responses of the paravertebral muscles during static contractions can be reliably measured by NIRS (Kell et al. 2004).

Sensitivity of NIRS to low-level changes in tissue oxygenation facilitates its application in many research areas. Tissue oxygenation, measured by NIRS, significantly decreased during brief, low-levels of static muscle contraction (Murthy et al. 1997). Kouzaki and co-workers (2003) showed that even during low-level sustained contraction, local circulation is modulated by the alternate muscle activity of knee extensor synergists. Similarly, McGill et al. (2000) assessing the level of back muscle oxygenation during prolonged isometric contractions observed that tissue oxygenation in the lumbar extensor musculature is reduced as a function of contraction intensity, even at levels as low as 2% of MVT.

NIRS and other techniques

NIRS has been used along with other common methods of measuring tissue oxygenation. Van Beekvelt and colleagues (2001) compared the mVO_2 and mBF obtained by NIRS to those of the Fick method and plethysmography. They suggested that NIRS is an appropriate tool to provide information about local mVO_2 and mBF because both place and depth of the NIRS measurements reveal local differences that are not detectable by the more established, but also more global, Fick method. De Blasi and colleagues (1994) have also reported similar findings. NIRS has also been compared with functional MRI, and it was found that NIRS and functional MRI similarly investigated cortical oxygenation changes in young and elderly subjects (Mehagnoul-Schipper et al. 2002).

NIRS has been applied as a complementary or primary tool beside other instruments to investigate human muscle characteristics. Boushel et al. (1998) reported that the oxygen kinetics of forearm flexor muscles measured by NIRS closely reflected the exercise intensity and the metabolic rate determined by MRS (Boushel et al. 1998). Similarly, Miura and associates (Miura et al. 1998) demonstrated that exercise capacity, with respect to both working muscle deoxygenation and ventilation, could be evaluated in detail by the concomitant use of NIRS and expired gas analysis.

Application of NIRS and EMG as two complementary assessing tools could conveniently disclose many of physiologic phenomenon of human muscle in health and disease. Praagman and colleagues (2003) investigated the relationship between local oxygen consumption in mVO_2 measured by NIRS and force or muscle activity measured by EMG and found a linear relationship between EMG signals and mVO_2. They suggested that load sharing is independent of force level and that next to EMG, VO_2 can be used for the validation of musculoskeletal models. Similar relationships between EMG and NIRS were reported by other groups (e.g., Alfonsi et al. 1999; Miura et al. 2000). However, the NIRS-EMG correlations were not strong in all conditions. Albert and co-workers (2004) examined the efficacy of EMG and NIRS in assessing erector spinae muscle activity during the Biering-Sorensen Back Muscle Endurance test. They observed that muscle oxygenation and median frequency were moderately related.

However, NIRS reliability has not been proved in all related studies. For instance, MacDonald et al. (1999) compared femoral blood gases and muscle NIRS at exercise onset, and found that NIRS does not provide a reliable estimate of Hb and/or oxygen saturation as reflected by direct femoral vein sampling (MacDonald et al. 1999). Hicks and associates (1999) also investigated the relationship between tissue oxygenation measured by NIRS and forearm muscle blood flow measured by Doppler ultrasound during isometric contractions at 10 and 30% MVT under conditions of normoxia and hypoxia (14% inspired oxygen). They concluded that although NIRS provides an indication of muscle oxygenation during isometric exercise, the conflicting findings in their study for NIRS and direct venous blood sampling at 10% vs. 30% MVT suggest caution in the application of NIRS.

Clinical application

NIRS has been increasingly employed in clinical investigations within the last 20 years. Jobsis in 1997 brought the idea of monitoring cerebral and myocardial oxygen sufficiency and circulatory parameters by NIRS. Since then, NIRS technique has been applied in humans to detect different circulatory (McCully et al. 1994; Belardinelli et al. 1997; McCully et al. 1997; Burnett et al. 2000, De Blasi et al. 2005), metabolic (Bank and Chance 1997; Belardinelli et al. 1997) and even genetic disorders (Lynch et al. 2002). In this section, NIRS application has been categorized and presented based on different parts, systems and disorders of the human body.

Cerebral conditions

The study of cerebral oxygenation using NIRS has become very popular compared to the study of other parts of the human body. The application of NIRS for investigating cerebral oxygenation has been extensively reviewed (Brazy 1991; Elwell et al. 1993; Benaron and Stevenson 1994; Cooper et al. 1994; Bernert et al. 1995; Bloom et al. 1996; Tamura et al. 1997; Dujovny et al. 1998; Boas et al. 2004; Edmonds et al. 2004; Aslin and Mehler 2005; Gratton et al. 2005).

NIRS has been a reliable and reproducible method for the evaluation of cerebrovascular reactivity and could be used for the assessment of patients with cerebrovascular disease (Totaro et al. 1998). For instance, brain tissue oxygenation in sickle cell disease patients were compared with that of healthy children, and it was demonstrated that abnormal oxygen saturation levels in sickle cell disease patients correlated with the severity of their clinical manifestations (Raj et al. 2004). Measuring cortical and subcortical oxygenation in healthy subjects and patients with a mono-hemispheric lesion in the middle cerebral artery territory, Vernieri and colleagues (1999) demonstrated that transcranial Doppler sonography mainly tests subcortical changes in the middle cerebral artery flow, while NIRS was sensitive to cortical arterioles and capillary blood flow modifications.

NIRS can also be applied to follow up the therapeutic effects of medications used in cerebral diseases. Vas et al. (2002) using NIRS and transcranial Doppler sonography methods have shown the cerebral perfusion-enhancing and parenchymal oxygen extraction-increasing effects of Vinpocetine (a medication for improving cerebral blood flow) in subacute ischaemic stroke patients. NIRS application has also been suggested for early detection of neurological injuries and issues of rehabilitation after paediatric cardiac extracorporeal membrane oxygenation (Golej and Trittenwein 1999).

Cardiovascular conditions

*Peripheral vascular disease***:** NIRS has been broadly used to detect peripheral vascular disease (PVD) and to assess the therapeutic effect of surgical or pharmacological treatments for PVD (Cheatle et al. 1991; Casavola et al. 1999). Cheatle et al. (1991) measured oxygen consumption and reoxygenation during and after a period of tourniquet-induced ischaemia in the calf muscles of 17 patients with PVD and 21 normal subjects. The results showed that oxygen consumption was reduced in PVD. In another study, the time constant of recovery of oxygen saturation was significantly higher in the PVD group (both legs) compared to normal subjects, and the values were more significant in their bad legs (McCully et al. 1994). Similar findings have been reported by other groups (Kooijman et al. 1997; Kragelj et al. 2001; Jarm et al. 2003; Wolf et al. 2003).

Intermittent claudication: NIRS can be used to accurately assess the severity of Intermittent claudication (Komiyama et al. 1994). NIRS-measured O_2Sat, could provide a different and more appropriate end point for diagnosis and monitoring of the management of patients with peripheral arterial disease, and may offer additional insight into the pathophysiology of exercise-induced muscle ischemia and its recovery (Comerota et al. 2003). Komiyama and colleagues (1994) used NIRS to measure the relative changes in HHb, HbO_2 and THb in the calf muscle of 19 claudicants during a treadmill-walking test and the subsequent recovery period. They found that after medical treatment in patients, recovery time of HbO_2 decreased in ten cases, increased in seven cases and remained unchanged in two cases, while pressure studies did not

facilitate the assessment of changes in the severity of claudication. This has been supported by other studies (Komiyama et al. 2000; Seifalian et al. 2001).

Heart failure: NIRS can detect impaired skeletal muscle O_2 delivery in patients with heart failure. Wilson et al. (1989) observed that during exercise, normal subjects exhibited an initial increase in Hb-Mb oxygenation (measured by NIRS), followed by a progressive decrease in oxygenation at the peak exercise workload. In contrast, patients exhibited an initial decrease in Hb-Mb oxygenation with the first workload, followed by a progressive further decrease at a peak exercise workload. In addition, at all exercise loads, Hb-Mb oxygenation was significantly less in the patients compared to the normal subjects. These findings supported their hypothesis and suggested that NIRS can detect impaired skeletal muscle oxygen delivery in patients with heart failure. Hanada and colleagues (2000) reported similar findings.

Ischemic heart disease: Perko and Bay-Nielsen (2002) investigated the changes in myocardial oxygenation in patients with ischaemic heart disease during surgical revascularisation. They found that pre-revascularisation values of all spectroscopy parameters were 40-50% lower in the myocardium than in the control group. After successful revascularisation O_2Sat, HbO_2, and THb in the myocardium increased by 20, 29, and 18%, respectively. Therefore, NIRS could be applied in an immediate assessment of postoperative changes in myocardial perfusion and oxygenation.

Microvasular dysfunction: NIRS has been successfully employed to investigate microvascular dysfunctions such as sepsis and septic shock. Using this technique, De Blasi and colleagues (2005) observed that patients with sepsis had significantly higher tissue blood volume and lower compliance than the healthy control group. Therefore, they concluded that septic shock changes microvascular muscle function and regulation.

Biochemical composition of human peripheral arteries has also been investigated with NIRS (Salenius et al. 1998), and it has been suggested that NIRS can provide reliable histochemical information about peripheral and coronary arteries. This may help to

identify rupture-prone plaques before the onset of symptoms and allow aggressive and directed intervention (Salenius et al. 1998).

Musculoskeletal disorders

Chronic muscle pain: Effects of exercise and ischemia on tissue oximetry and cytochrome have been studied in normal subjects, patients with chronic limb pain, and patients with mitochondrial myopathies (Delcanho et al. 1996; Chelimsky et al. 1997). Delcanho and co-workers (1996) have suggested that patients with chronic muscle pain have a slower intramuscular reperfusion during the recovery phase after sustained isometric contractions.

Back pain: Kovacs and associates (2001) studied the kinetics of oxygen consumption of the erector spinae muscle while simultaneously monitored motion characteristics of the muscle to determine whether oxygen consumption was different between healthy and low back pain subjects. Their results showed significant differences in oxygen use but not blood volume between healthy and low back pain subjects with muscular-based disorders.

Scoliosis: To investigate the extent of stress, Miyake and co-workers (2003) measured oxygen dynamics in both sides of the paraspinal muscles of patients with degenerative lumbar scoliosis. There was a significant difference in the average recovery time for HHb between the patient and control groups. In addition, the recovery time on the concave side was faster than that on the convex side. Their results supported the theory that stretching the muscles at the convex side results in chronic increases in the intramuscular compartment and reduced muscular blood flow.

Compartment syndrome: NIRS could be useful as a non-invasive diagnostic tool for the evaluation of patients suspected of having chronic compartment syndrome of the leg. Several groups have applied NIRS for the diagnosis of chronic compartment syndrome (Breit et al. 1997; Mohler et al. 1997; Van Den Brand et al. 2004). For instance, Mohler et al. (1997) studied the intramuscular pressure and the relative oxygenation in the

anterior compartment of the leg in eighteen patients who were suspected of having chronic compartment syndrome, as well as in ten control subjects before, during, and after exercise (dorsi- and plantar flexions). During exercise, maximum relative deoxygenation in the patients who had chronic compartment syndrome was significantly greater than that in the patients who did not have chronic compartment syndrome and that in the control subjects. Moreover, the time interval between the cessation of exercise and the recovery of the pre-exercise resting level of oxygenation was significantly longer for the patients who had chronic compartment syndrome than for the patients who did not have chronic compartment syndrome and the control subjects (Mohler et al. 1997).

NIRS application during exercise

NIRS is being increasingly used to monitor muscle oxygenation during various types of exercise. Table 1 shows some of the studies, which reviewed the NIRS application in exercise science. Sport scientists, using NIRS, have studied various physiological aspects of human skeletal muscles. The literature related to the application of NIRS in sport science is discussed briefly below.

Table 1. Some of the recent articles that have reviewed the application of NIRS in sport and exercise science.

Topic	Comments	Author(s)
Near-infrared spectroscopy for monitoring muscle oxygenation	NIRS has demonstrated utility for monitoring changes in muscle oxygenation and blood flow during submaximal and maximal exercise.	Boushel and Piantadosi (2000)
Near-infrared spectroscopy: what can it tell us about oxygen saturation in skeletal muscle?	NIRS remains promising for studies of skeletal muscle, but a better understanding of the method is needed.	McCully and Hamaoka (2000)
Monitoring tissue oxygen availability with NIRS in health and disease	NIRS application can be fruitful in the study of occupational syndromes and a variety of diseases.	Boushel et al. (2001)
Accuracy limits in the determination of absolute optical properties using time-resolved NIR spectroscopy	Temporal resolution of the order of 10 ps is necessary for quantifying the absorption and reduced scattering coefficient of diffuse media with accuracy better than 5% using curve fitting methods.	Ntziachristos and Chance (2001)
The use of near infrared spectroscopy in sports medicine	NIRS can objectively evaluate muscle oxidative metabolism in athletes and its modifications following potential therapeutic strategies and specific training programs.	Quaresima et al. (2003)
Principles, techniques, and limitations of near infrared spectroscopy.	NIRS is a noninvasive and relatively low-cost optical technique that is becoming a widely used instrument for measuring tissue O2 saturation, changes in hemoglobin volume and, indirectly, brain/muscle blood flow and muscle O2 consumption.	Ferrari et al. (2004)
Muscle oxygenation trends during dynamic exercise measured by near infrared spectroscopy.	There is a strong correlation between the lactate (ventilatory) threshold during incremental cycle exercise and the exaggerated reduction in muscle oxygenation measured by NIRS.	Bhambhani (2004)

Gender

The issue of difference in oxygen kinetics during exercise between men and women has not been properly addressed, and in a few presented reports, dissimilar results have been reported. For instance, in one study (Bhambhani et al. 1998) the rate of decline in muscle oxygenation for a given increase in VO_2 was independent of exercise mode and gender. Whereas, in another study by Bhambhani and colleagues (1999), a significantly higher muscle oxygenation was demonstrated at different exercise intensities in women compared to men (suggesting lesser deoxygenation at the same relative exercise intensity). Therefore, it can not be concluded that the gender difference in muscle oxygenation is a true physiological phenomenon or it is an artifact of the NIRS technique (Bhambhani et al. 1999). Hence, more studies are warranted to examine this issue taking into account other factors such as body mass index.

Blood lactate and oxidation

To evaluate whether NIRS can be used to assess metabolic capacity of muscles, Ding and colleagues (2001) studied the changes in muscle oxygenation and blood lactate concentration of 18 elite male athletes and eight healthy young men during cycling at different intensities. Their results showed a correlation between muscle oxygenation and blood lactate concentration at various exercise intensities (Ding et al. 2001). Similarly, Murakami et al. (2000) measured oxygen consumption of non-exercising skeletal muscles at rest and after aerobic exercise, and observed significant correlations between the increase in VO_2 and the increase in lactate concentration. Miura and colleagues (2000) reported similar findings to those of Murakami and associates(2000).

Kinetics of muscle oxygenation

NIRS has been successfully employed to measure muscle oxygenation and blood flow during rest and exercise (De Blasi et a. 1993, De Blasi et al. 1996). This technique has also provided insights into the kinetics of oxidative metabolism during exercise transitions (Grassi et al. 2003; Volianitis et al. 2003). Grassi and colleagues (2003) observed a constant muscle oxygenation during the initial phase of the on-transition,

which indicated a tight coupling between increases in oxygen delivery and utilization. Volianitis and co-workers (2003) demonstrated a significant reduction in arm blood flow during transition from arm exercise to arm + leg exercise.

Because of the significant correlation with regulatory metabolites (ADP and PCr) of oxidative phosphorylation, oxygen decline rate (determined by NIRS) in immediate post-exercise ischemia can be applied for the quantitative evaluation of localized muscle oxidative metabolism (Hamaoka et al. 1996). To evaluate whether NIRS could be used to assess metabolic capacity of muscles, Ding and associates (2001) assessed two distinctive variables (the rate of recovery of muscle oxygen saturation and the relative value of the effective decrease) abstracted from a curve of changes in muscle oxygenation. They suggested that these two variables could be used to characterise muscle oxidative metabolism during human body movement.

NIRS has also been successfully applied to find correlations between different parameters involved in muscle oxygenation. Kawaguchi and colleagues (2001) found a marked positive correlation between HHb and VO_2, and a significant negative correlation between O_2Sat and VO_2 in vastus lateralis muscle during an exercise tolerance test on a bicycle ergometer. In addition, they observed a negative correlation between VO_2 and HbO_2, and no correlation between THb and VO_2. Their results suggested that kinetics of peripheral muscle oxygenation reflect systemic VO_2. Im and colleagues (2001) observed similar findings and suggested that during cross-country ski skating, the strong relationship of oxygen desaturation percentage to whole body VO_2 may be attributed to oxygen dissociation in the capillary bed of the muscle to meet aerobic energy demand, and it was independent of blood flow dynamics.

Regional difference in muscle oxygenation

NIRS has been demonstrated to be a useful method to detect the regional differences of oxygen status in the muscle. Boushel and associates (2000) used NIRS and the tracer indocyanine green to quantify regional tissue blood flow during exercise in humans and suggested that due to its high spatial and temporal resolution, the technique could be

useful for determining regional blood flow distribution and regulation during exercise in humans. Similarly, Miura and colleagues (2001) observed greater oxygen saturation and blood volume changes (measured by NIRS) in the distal portion of gastrocnemius muscle during exercise compared to the other parts of this muscle. Furthermore, Hiroyuki and colleagues (2002) observed that the pattern of deoxygenation between vastus lateralis and lateral head of gastrocnemius during treadmill walking and running was somewhat different and that the muscle oxygenation level was associated with pulmonary VO_2. Similar observations in other studies (Azuma et al. 2000; Ogata et al. 2002), further supported the effectiveness of NIRS in detecting regional differences in oxygenation.

Comparison between different types of exercise

NIRS has been employed to compare muscle oxygenation in different types of exercise. For instance, Bhambhani and co-workers (2001) compared the acute cardiorespiratory responses and muscle oxygenation trends during incremental cycle exercise to exhaustion with those observed during 30 s and 45 s Wingate tests in healthy men and women, and found a significantly higher oxygen uptake during the incremental test compared to the Wingate test. Nioka and colleagues (1998) also used NIRS to compare the pattern of oxygen consumption in aerobic (VO_2max test) and anaerobic (Wingate test) exercise by monitoring oxygen concentration of the vastus lateralis muscle at maximum intensity. Their findings revealed that in the Wingate test, the deoxygenation reached approximately 80% of the established maximum value, while the VO_2max test resulted in approximately 36% deoxygenation. There was no delay in the onset of deoxygenation in the Wingate test, while in the VO_2max test, deoxygenation did not occur under low intensity work (Nioka et al. 1998).

Training effect and adaptations

Although the effect of training on muscle oxygenation kinetics had been studied using different methods, NIRS may provide a more specific and detailed information on training-induced adaptation(s), if any, in regional tissue oxygenation. Based on the data obtained by NIRS, Neary and colleagues (2002) suggested that a significant

improvement in VO_2max induced by short-term endurance training in well-trained cyclists was due primarily to central adaptations, whereas a simulated 20 km time trial performance was enhanced due to localized changes in muscle oxygenation.

Moreover, Usaj (2001) examined the influence of training on the oxygenation status of a forearm muscle during submaximal isometric contraction. The training effect, which was represented as the difference between the duration of muscle contraction before and after the training period, was significantly correlated with relative oxygen saturation and with relative concentration of deoxygenated haemoglobin. The training-induced adaptation in blood lactate may also influence muscle oxygen saturation measured by NIRS during mild- to hard-intensity exercise (Costes et al. 2001). Therefore, NIRS can be used as a non-invasive monitoring of training-induced adaptations (Costes et al. 2001).

Muscle fatigue and soreness

NIRS has rarely been used to study the long-term effects of vigorous exercise on muscle oxygenation. The structural alterations, observed within muscle after strenuous exercise, may restrict local blood flow and increase the diffusion distance of oxygen to muscle fibres. Walsh and colleagues (2001) evaluated the effects of eccentric exercise on muscle oxidative function and demonstrated that eccentric cycling, although causing delayed onset of muscle soreness, did not negatively affect skeletal muscle oxidative function measured by NIRS.

On the other hand, simultaneous recordings of EMG, mechanomyography, and NIRS showed that the restriction of blood flow due to high intramuscular mechanical pressure was one of the most important factors in muscle fatigue in the lower-back muscles (Yoshitake et al. 2001). In addition, ischemia leading to a 7% or greater reduction in muscle oxygenation decreased muscle force production in the forearm extensor muscle (Murthy et al. 2001).

Effects of nutritional and metabolic agents on muscle oxygenation

Application of NIRS has provided new insights into the effects of different metabolic factors on oxidative metabolism. For example, NIRS application revealed the important synergistic role of nitric oxide and prostaglandins for skeletal muscle vasodilatation and hyperaemia during muscular contraction (Boushel et al. 2002). In another study, CO_2 influenced blood flow to active skeletal muscle, although its effect was smaller than for the brain (Nielsen et al. 2001). Additionally, NIRS application revealed that the enlarged blood-buffering capacity after infusion of sodium bicarbonate attenuates acidosis and arterial desaturation during maximal exercise (Nielsen et al. 2002).

NIRS application could also reveal the effects of nutritional factors, if there is any, on muscle oxygenation. Thompson and colleagues (1996), using NIRS and MRS, studied the effect of a relatively low dose of creatine on skeletal muscle metabolism and oxygen supply in a group of training athletes. Their findings showed that oral creatine supplementation at 2 g daily has no effect on muscle creatine concentration, muscle oxygen supply or muscle aerobic or anaerobic metabolism during endurance exercise.

Advances in NIRS technology

Thank to advances in both optical and electronic technologies, NIRS instrumentation and application are being improved continuously. There are four key components for each NIRS system, including the source of electromagnetic energy for the interrogating beam and the number of wavelengths used; the means with which the energy is delivered to and collected from the object; the method used for processing the received signals; and the type of detector (Rolfe 2000). Therefore, the desired overall performance of a system could be achieved by the careful adjustment of each of these elements.

For instance, a modular NIRS system for clinical measurement of impaired skeletal muscle oxygenation was designed few years ago (Wariar et al. 2000). The authors claimed that this is a valid system design for the precise, accurate, and sensitive

detection of changes in bulk skeletal muscle oxygenation, which can be constructed economically and can be used diagnostically in patients with disorders of skeletal muscle energy metabolism (Wariar et al. 2000). Another group (Takaishi et al. 2002) presented a method that reorders NIRS parameters against crank angle. They demonstrated that this method serves as a useful measure in providing additional findings of circulatory dynamics and metabolic changes in a working muscle during pedalling exercise.

In humans, NIRS was initially used to monitor tissue oxygenation at one particular region. Then, due to the interest in the simultaneous application of NIRS measurements at more than one anatomical site, the two-channel or multichannel instrumentation was emerged. The further need for the simultaneous examination of several regions of tissue led to developments in NIR imaging (Rolfe, 2000).

NIR imaging or mapping has been developed using different NIRS systems (e.g. continuous wave, time domain and intensity modulated techniques). However, the lack of pathlength determination limits the accuracy of the results from continuous wave systems. Imagent (ISS Inc., Urbana, IL) is a new device that uses frequency domain system to measure the absolute values of HbO_2 and HHb concentration maps in tissue. Multichanell TRS systems have also been developed to measure the absolute values of HbO_2 and HHb (Ferrari et al. 2004). However, NIRS multichannel systems have some limitations, including poor spatial resolution, difficulty with precise anatomical localization, and relatively poor penetration and localization depth (Ferrari et al. 2004).

Summary

As described earlier in this chapter, muscle damage may alter the pattern of muscle oxygenation. NIRS applications have demonstrated reliable and valid measurements in clinical assessments and follow up treatments, as well as in exercise physiology. Although NIRS alone is an effective tool to detect some of the disorders in humans, it would be more effective when complemented with other techniques. In the experiments presented in this thesis, NIRS was employed to monitor the changes in muscle

oxygenation following sessions of concentric or eccentric contractions. The following four chapters will present these experimental studies.

Chapter 3

Monitoring Muscle Oxygenation and Blood Flow after Eccentric Exercise-Induced Muscle Damage using Near Infrared Spectroscopy

As co-authors of the paper "Ahmadi S, Sinclair PJ, Foroughi N, Davis GM. Monitoring Muscle Oxygenation and Blood Flow after Eccentric Exercise-Induced Muscle Damage using Near Infrared Spectroscopy. *Applied physiology, nutrition and metabolism* (In review 2007)", we confirm that Sirous Ahmadi has made the following contributions:

- conception and design of the research
- data collection
- analysis and interpretation of the findings
- writing the paper and critical appraisal of content

PJ Sinclair…..……...……Date:……

N Foroughi ……..Date:……

GM Davis …….……..Date:……...

Abstract

Background: Delayed onset muscle soreness and impaired neuromuscular function are the common consequences of vigorous eccentric exercise that induces muscle damage. We investigated the hypothesis that strenuous eccentric exercise (EE) might impair muscle oxygenation and muscle blood flow in healthy adults. **Method:** Ten healthy males performed a bout of 70 maximal elbow flexion eccentric contractions. Before and after EE on day 1 and over the next six days, maximum voluntary isometric torque (MVT), plasma creatine kinase activity (CK) and the changes in muscle oxygen saturation, blood flow and oxygen uptake (using near infrared spectroscopy) within biceps brachii were assessed. **Results:** MVT decreased while muscle soreness and CK increased significantly ($p<0.05$) after EE, consistent with EE's effectiveness to evoke muscle damage. Mean resting oxygen saturation increased by 22% after acute EE, and remained elevated by 5-9% for the following 6 days. During isometric contractions, significant decreases were observed in oxygen desaturation and re-saturation kinetics after EE and these declines persisted over the following 6 days. Both muscle blood flow and oxygen uptake increased significantly after acute EE, but recovered on the next day. Conclusion**s:** This study revealed some prolonged alterations in muscle oxygenation at rest and during exercise after EE, which might be due to a decrease in oxygen consumption, an increase in oxygen delivery, and/or a combination of both.

Key Words: Biceps Brachii, Oxygen Saturation, Delayed Onset Muscle Soreness, Creatine Kinase, Oxygenation Kinetics.

Introduction

Delayed onset muscle soreness (DOMS) and impaired muscle function are the common consequences of unaccustomed eccentric exercise (EE). Some of morphologic and metabolic alterations in the muscle that are presumed consequences of EE include: (i) a prolonged decrease in muscle glycogen concentration (O'Reilly et al. 1987); (ii) changes in capillary luminal shape or area (Kano et al. 2004); (iii) impairment of muscle microcirculatory flow (Kano et al. 2005); (iv) myofibrillar damage along the Z-band (Friden et al. 1984) ; and, (v) mitochondrial swelling and increased intramuscular pressures (Friden et al. 1983). Eccentrically-induced muscle damage may cause perturbations in resting metabolic rate up to 48-hr post-exercise (Dolezal et al. 2000). Furthermore, long term effects of EE upon muscle metabolism have been observed (Rodenburg et al. 1994), such as elevation in the ratio between inorganic phosphate and phosphocreatine in muscles measured by ^{31}P magnetic resonance spectroscopy at rest for up to a few days after EE.

Increased intramuscular pressures (Friden et al. 1983), vasodilatation and increased water content of the muscle after EE, may change the pattern of local blood flow and muscle oxygenation. In addition, possible changes in muscle fibre recruitment after EE-induced muscle damage may also influence the pattern of tissue oxygenation. Lieber and Friden (1988) demonstrated that there was a selective damage of fast-twitch glycolytic fibres after EE. After such damage, muscles may recruit fewer fast-twitch and greater slow-twitch fibres for a given task. Slow-twitch fibres possess greater oxidative capacity, and their recruitment for a given task may result in higher oxygen consumption compared to fast twitch fibres. Additionally, muscle damage results in increased mithochondrial Ca^{2+} concentration (Duan et al 1990). This may result in an impairment of muscle respiration and ATP production (Wrongemann and Pena 1976).

The effects of EE on muscle oxygenation and muscle blood flow (mBF) in humans have not been thoroughly investigated. Moreover, in the few studies that have assessed changes in muscle oxygenation and/or mBF after EE, disparate results were reported. For instance, Kano and co-workers (2004) observed significant alteration in capillary

luminal shape and area for up to 3 days after eccentric exercise in rodents. In another animal study, Kano et al. (2005) also demonstrated that downhill running impaired muscle microcirculatory flows as well as the balance between oxygen delivery and consumption at the onset of such exercise. Furthermore, our previous work (Ahmadi et al., in press) revealed significant increases in oxygen desaturation and resaturation amount and rate for up to four days following an exhaustive session of downhill walking. In contrast, Walsh and co-workers (2001) did not report any significant changes in oxygen utilization or local oxygen transport after a 30-min session of eccentric cycling in humans. Similarly, Laaksonen and colleagues (2006) did not find any significant changes in oxygen uptake after exhaustive stretch-shortening cycle exercise, although they observed a significant increase in mBF.

These contradictory findings could be due to the methodological differences in inducing muscle damage and consequently in the magnitude of damage, if there was any. Additionally, the application of different techniques to assess muscle oxygenation and blood flow could also result in discrepancies in findings. However, the disparity amongst previous studies, suggested that further investigation into the effect of EE upon muscle oxygenation and associated measures was warranted. Accordingly, this study investigated the hypothesis that a session of unaccustomed eccentric contractions of elbow flexors might alter/impair muscle oxygenation and mBF within biceps brachii.

We employed near infrared spectroscopy (NIRS) to monitor the pattern of muscle oxygenation and blood flow of biceps brachii before and for six days after a session of EE. NIRS was first described by Jobsis (1977), as a simple and non-invasive tool to estimate the proportion of regional oxygenated and deoxygenated haemoglobin/myoglobin(Hb/Mb) within skeletal muscles of healthy humans, and in those with underlying pathophysiology (McCully et al. 1994). The theory behind NIRS methodology and its application have been well explained previously (e.g. Boushel et al. 2001; Quaresima et al. 2003). The reproducibility, feasibility, and sensitivity of NIRS measurements have also been previously documented (e.g. Hampson and Piantadosi 1988; Van Beekvelt et al. 2001). NIRS can be employed for the measurement of oxygenated (HbO_2), deoxygenated (HHb) and total (THb)

haemoglobin, as well as tissue oxygen saturation (O_2Sat), muscle blood flow (mBF) and muscle oxygen consumption (mVO_2; Quaresima et al. 2003). The blood volume assessed by NIRS is different from the muscle blood flow, but one can utilise it as a relative index of local blood circulation (Kouzaki et al. 2003). In addition to some parameters that have been employed in previous works (Walsh et al. 2001; Laaksonen et al. 2006), some new parameters of muscle oxygenation were assessed and monitored during isometric contractions at given intensities after downhill walking to further reveal changes in muscle oxygenation, if any.

Methods

Subjects: Ten healthy males (age 25 ± 4 yr, body mass 73.1 ± 9.8 kg, stature 173.2 ± 7.0 cm; mean ± SD), who had not participated in any regular muscular upper body exercise training (i.e. at least once per week for more than 30-mins) for 12-months prior to commencing the study, took part in the experiment. In this preliminary study, the authors employed a "sample of convenience", based on results from previous investigations (Neary et al. 2002; Laaksonen et al. 2006), which we estimated would show a reasonable chance of post exercise changes in muscle oxygenation parameters, if there were any to be observed. The Human Research Ethics Committee of the University of Sydney approved the study. All subjects were informed of the purpose, nature, and potential risks of the study, and gave their written informed consent to participate. The subjects were in healthy physical condition with no signs or symptoms of neuromuscular disease. They were not under pharmacological treatments and followed a normal diet. All subjects were requested to abstain from any exercise involving arm muscles for the duration of the study.

Study design: Testing sessions were performed over a 7-day period. The biceps brachii of the non-dominant arm performed EE (Exercise), whereas the biceps brachii of the dominant arm performed no exercise (Control). Changes in criterion measures were compared between Control and Exercise arms.

Eccentric exercise protocol: The exercise protocol used in this investigation was modified from previous studies that had been designed to induce muscle damage (Clarkson et al. 1992; Felici et al. 1997; Sbriccoli et al. 2001) and has been described in one of our in-press studies (Ahmadi et al. 2007a). Subjects were habituated to the equipment and performed some isometric contractions similar to those that they would perform during testing days. On the first day (day 0), subjects performed two sets of 35 maximal voluntary EE contractions (4-s of contraction and 12-s of passive recovery) with the elbow of their non-dominant arm placed in an isokinetic apparatus (Biodex System 2, Biodex, USA). Subjects were encouraged both verbally and visually (via force output displayed on a computer monitor), to maximally resist elbow extension movements in which the arm was forcibly extended from an elbow-flexed (on average 50°) to an elbow-extended (on average 170°) position at a preset angular velocity of 30 $deg \cdot s^{-1}$. The apparatus brought the subject's arm back to the elbow-flexed position after each eccentric contraction (passive recovery). The two EE sets were separated by a 5-min recovery interval.

Measurements: Before and 30-min after the EE session on day 0 and for the next 6-days, the following measurements were made on Exercise and Control arms: skinfold thickness, plasma creatine kinase, elbow range of motion, upper arm circumference, individual's perception of muscle soreness, maximum voluntary torque, and NIRS-obtained parameters. It should be noted that the order of performing the assessments (as described below) were the same in all sessions.

Skinfold thickness: Adipose tissue thickness may have a substantial confounding influence on in vivo NIRS measurements (Van Beekvelt et al. (Van Beekvelt et al. 2001). Therefore, in this study skinfold thickness was assessed to determine any relationship between these and resting O_2Sat, mVO_2 or mBF. On the first day before commencing the experiment, the thickness of the skin at the middle portion of biceps brachii (where the NIRS probe was to be placed), was measured using Harpenden skinfold callipers (John Bull; British Indicators, London, UK). Skinfold measurements were performed while subjects stood with arms hanging relaxed. Each measurements was performed 3-times until all three values obtained were within 5% of the first

measurement. The average for the outcomes of three measurements was used for statistical analysis.

Plasma creatine kinase (CK) activity: CK activity was measured on day 0 before and after EE, and on days 2, 4, and 6 at the beginning of each isometric exercise session. At each sampling time, about 5-ml of venous blood was withdrawn from the antecubital vein, centrifuged for 10-min to extract plasma samples, and analysed for CK activity within 24-hr. Plasma CK activity was assayed spectrophotometrically at 37°C using CK-NAC reagent kits (Thermo Electron Corp., Woburn, MA, USA). Normal resting values of CK using this method are ≤175 units per litre ($U \cdot L^{-1}$) for men (Burtis and Ashwood 1994). Each plasma sample was assayed at least twice, until two assays were within 10% of the lower value. The mean of the two values was used for statistical analyses.

Elbow range of motion (ROM): Subjects were instructed to stand beside a whiteboard in a relaxed position with their investigated arm relaxed (extended position). At this time, the investigator marked the locations of the shoulder (Acromion), elbow (Olecranon) and wrist (Styloid process) on the whiteboard and measured the angle using a goniometer. The subject then flexed his forearm while the elbow and shoulder joint positions were held constant by another investigator. The new position of wrist was marked again on the whiteboard (flexed position) and the angle was measured. ROM was assessed as the angle between extended and flexed positions. This was repeated 3-times and the average of the 3 ROM measurements was used for statistical analyses.

Arm circumference (CIR): CIR was measured at 4, 6, 8, and 10 cm above the elbow joint, while allowing the arm to hang down by the side. These measurements were repeated 3 times at each anthropometrical location and the average of the three trials for all antropometrical locations was used for statistical analyses.

Perception of muscle soreness (SOR): A subjective rating of SOR was performed during each session using a 7-point categorical scale, where 1 corresponded to “no pain” and 7 to “very, very painful”. While standing, the subjects were instructed to gently

palpate their upper arm during full range of motion biceps curls and then choose the number that corresponded to their perceived level of soreness (Sayers et al. 2000).

Near infrared spectroscopy measurements: NIRS measurements were obtained using a commercially available dual-channel frequency-domain system, (ISS OxiplexTS Oximeter, Model 96208, ISS Inc., Champaign, IL, USA) with identical NIRS probes for each channel (we used only one channel in this study). The NIRS system comprised an optical detector and a light source deploying wavelengths of 690 and 830-nm. Each sensor probe had four emitter positions (eight emitters) with emitter-detector distances ranging from 1.5-cm to 5.0-cm.

The NIRS system was calibrated using standard calibration blocks provided by the manufacturer (ISS Inc.). A NIRS probe was attached to the middle part of subject's biceps brachii muscle longitudinally. To prevent variations in placement of the NIRS emitter-detector, the angle and location of the probes were held constant during the test using double-sided adhesive tape. Similarly, the position of the NIRS probe was noted to the nearest millimetre and identified with a marker, to ensure identical placement on each subject for all testing sessions. A light-impermeable cloth covered the probe to reduce room light interaction with near infrared signal. This frequency-domain NIRS system provided a direct estimation of oxyhaemoglobin (HbO_2), deoxyhaemoglobin (HHb) and total haemoglobin (THb). Tissue oxyhaemoglobin saturation (O_2Sat), which is a commonly-derived parameter from NIRS studies, was the ratio of HbO_2 to THb (Boushel et al. 2001), and was calculated using the equation :

$$O_2Sat = (HbO_2/THb)*100$$

To estimate mBF at rest, two venous occlusions were applied above the belly of the biceps muscle (using a cuff air pressure inflated to 70-mmHg), each lasting 20-s with a 2-min recovery interval. mBF was then estimated by measuring the initial linear increase in THb (De Blasi et al. 1994, Kooijman et al. 1997; Boushel et al. 2000). Concentration changes of THb were expressed in micromolar per second, and converted into units of millilitre per minute per 100 millilitres of tissue, using an average Hb

concentration of 8.5 milimolar per litre for male subjects (Van Beekvelt et al. 2001). The molecular weight of Hb (1 mole Hb weighs 64.458 kg) and the Hb to oxygen ratio (1:4) were also taken into account (Kooijman et al. 1997; Van Beekvelt et al. 2001). mBF during rest was calculated as the average during the two venous occlusions.

Then, after a further 3-min of recovery, supra-systolic arterial occlusion (cuff air pressure inflated to 270-mmHg) was performed to estimate local muscle oxygen consumption (mVO_2). Arterial occlusion was continued until the O_2Sat reached a nadir lasting at least 30-s, usually after 5 to 8-min, then the cuff was released and after 5-min rest, subjects were prepared for the exercise trials. The initial linear decrease in HbO_2 was used to calculate mVO_2 (De Blasi et al. 1993, Kooijman et al. 1997; Van Beekvelt et al. 2002). The changes in HbO_2 given by the spectrophotometer are in micromolar. This can be further converted to millilitres O_2 per minute per 100 g tissue taking in account the following assumptions. The amount of oxygen that binds to haemoglobin (1 mole of Hb binds to 89.6 litres of oxygen, assuming STPD conditions) and the muscle density (1.04 kg per litre) was used to estimate mVO_2 (Kooijman et al. 1997; Van Beekvelt et al. 2002) .

O_2Sat reflects the dynamic balance between O_2 supply and O_2 consumption and is independent of the path length of near-infrared photons within the muscle tissue (Ferrari et al. 2004). Therefore, the major part of NIRS data presented within this paper was obtained from the changes in O_2Sat, collected at rest, during venous and arterial occlusions, and during isometric contractions at different intensities. However, we also compared the resting values of THb, HbO_2 and HHb pre-post EE and over seven days. In addition to the traditional variables associated with NIRS, we derived some additional measures to express the absolute volume and rate of change (kinetics) of muscle oxygen saturation during exercise (Ahmadi et al. 2007b). The changes in oxygen saturation during short-term isometric contractions with different intensities could provide important information about oxygen delivery and consumption after DOMS. Changes in muscle oxygen desaturation (ΔdeO_2Sat) were calculated as the difference in O_2Sat from resting levels to nadir during exercise. The rate of change in ΔdeO_2Sat from rest to nadir provided a measure of the oxygen saturation kinetics,

which we termed Tau-deO_2Sat (ΔdeO_2Sat divided by time from rest to nadir). Similarly, the muscle oxygen resaturation volume (ΔreO_2Sat) and kinetics (Tau-reO_2Sat) were calculated from the nadir of oxygen saturation during exercise to the highest point during recovery. Figure 1 provides a sample of NIRS O_2Sat measurements obtained within each assessment session..

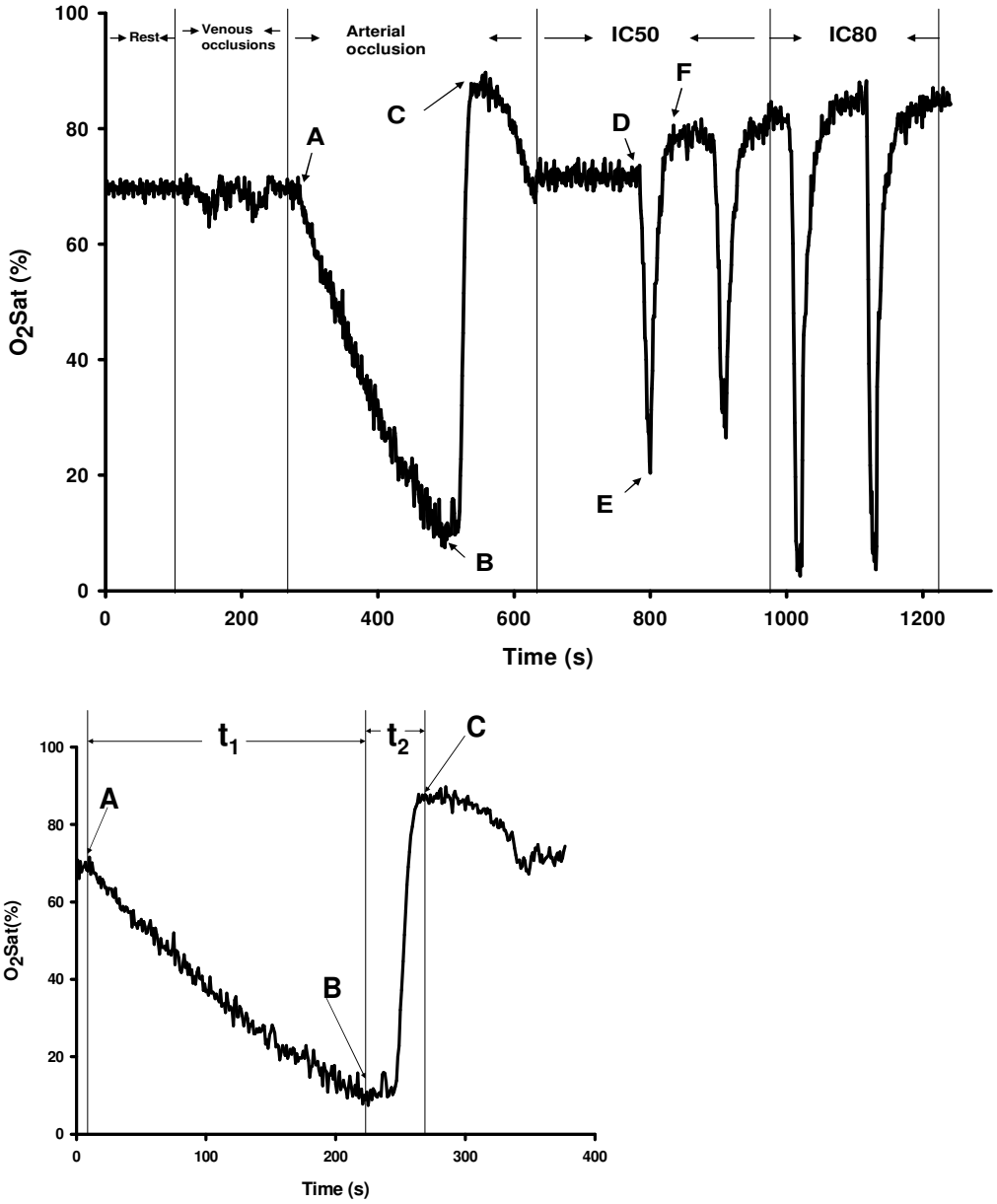

Figure 1. Top panel: A sample of NIRS O_2Sat measurements obtained within each assessment session. Bottom panel: A sample of NIRS O_2Sat trace obtained during super-systolic arterial occlusion. Some of the measurements and terms used within this study are graphically described herein. A - B = changes in oxygen desaturation (ΔdeO_2Sat) obtained during arterial occlusion. (A – B) / t_1 = the rate of change in ΔdeO_2Sat from rest to nadir (Tau-deO_2Sat). Similarly, C – B and (C – B) / t_2 represent ΔreO_2Sat and Tau-reO_2Sat, respectively. During isometric contractions, similar calculations using D, E, and F (Top panel) were made during 50% and 80% MVT (IC50 and IC80), respectively.

Isometric contractions: Maximum voluntary isometric contraction torque (MVT) was assessed on Exercise and Control limbs with the subject's elbow joint set to 90° and shoulder at 45° using the Biodex System. Three 5-s repetitions were performed with 2-min of recovery between each maximal effort. The highest value was taken to represent the 100% MVT, and was employed for statistical analyses.

After a further 5-min of recovery, isometric elbow flexions were assessed on Exercise and Control limbs at 50% of MVT (IC50). The highest MVT from the first day before EE was used to set IC50 for all subsequent sessions. The rationale for IC50 was to set an absolute muscle contraction force of moderate intensity between days before and after the EE stimulus. During each session subjects performed two isometric contractions at IC50, each lasting 20-s with 3-min of recovery between efforts.

Isometric elbow flexions were also assessed on Exercise and Control at 80% of MVT (IC80), whereby MVT was calculated for that session. The torque values for IC80, therefore, varied between days. The rationale for IC80 was to set a relative muscle contraction force (since MVT was assumed to fall on days after EE) of high intensity before and after eccentric exercise-evoking muscle damage. Two contractions were performed at IC80 of equal duration and recovery intervals as for IC50. Participants could observe their effort to reach and maintain the average exercise intensity requested by the investigator (50%, 80%, or 100% of MVT).

We chose the approach to assess muscle oxygenation during IC50 and IC80, to document any changes of oxygenation at the same ‘absolute’ force as well as at the same ‘day-relative’ force. It is also worthy to note that the IC50 and IC80 assessments were performed on every day in order to assess muscle oxygenation (using NIRS) during exercise of given intensities. However, on the first day only, on average 10-min after the last IC80, subjects performed EE contractions (as described in ‘Eccentric exercise protocol’). Then, subjects were rested in a comfortable position for 30-min when the pre-EE assessments were repeated. The experimental protocol measured one arm at a time and subjects were unaware which was going to be assessed first. However,

on day one after the pre-EE assessments on Control and Exercise limbs, subjects performed eccentric contractions with their Exercise arm, then after 30-min passive rest, their Exercise and Control arms were assessed, respectively (post-EE assessments). The experimental sessions were set at the same period every day (in the mornings) for each subject, and room temperature was set between 23°C to 25°C for all subjects.

Statistical analysis

A two-way repeated-measures analysis of variance (RM ANOVA) was employed to assess whether there were significant changes in dependent variables over time (eight assessments, with two assessments on day 1 before and after EE) and between arms (Control, Exercise). In these RM ANOVA analyses, the within-subjects factors comprised time (8 assessments), and the between-subjects factor was arm (Control versus Exercise). When a significant arm, time or arm-by-time effect was observed, univariate (one-way) RM ANOVA (main effect factor being time) was employed for each arm to test for day-day effects and within-day effects (i.e. two assessments on day 1 before and after EE). For CK, a one-way RM ANOVA was performed without an arm main effect, since this measure represents a circulating enzyme. Spearman correlation was used to measure the association between NIRS variables and skinfold thickness. Statistical significance for a meaningful change was set at the 95% confidence level ($p < 0.05$). All values were reported as mean ± standard error (SE).

Results

The two-way repeated-measures ANOVA (arm x time interaction effects) revealed significant changes in all indicators of muscle damage (i.e. MVT, ROM, SOR and CIR), and NIRS resting measures (i.e. O_2Sat, HHb, HbO_2, mVO_2, mBF). Similarly, the two-way RM ANOVA showed significant changes in Tau-deO_2Sat, ΔdeO_2Sat and ΔreO_2Sat during both IC50 and IC80. This means that there were time, arm or time by arm significant effects for the measured variables. Therefore, a one-way ANOVA was performed on all mentioned variables and revealed that there were not any significant

changes on the Control arm. In contrast, the univariate ANOVA revealed the following significant changes on the Exercise arm.

MVT: MVT decreased by 45% after EE, and remained significantly below the pre exercise value on day 0 (pre-EE) for the following 5-days (Table 1).

CK: CK activity increased by 18%, approximately one hour after EE, reached its maximum on day 4, and was still significantly higher than pre-EE values by day 6 (Table 1).

Table 1. Summary of the indirect indicators of muscle damage measured in this study.

		PRE	POST	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6
MVT	**Exercise**	59.7 ± 3.7	32.8 ± 4.8 *	40.7 ± 4.3*	41.6 ± 3.8*	48.4 ± 4.4*	49.6 ± 5.1*	53 ± 4.5*	53.7 ± 4.5*
(Nm)	**Control**	61.7 ± 3.0	57.9 ± 4.0	61.3 ± 3.5	63.3 ± 2.4	63.5 ± 2.9	65.1 ± 2.4	62.6 ± 3.5	67.0 ± 3.1
SOR	**Exercise**	1.0 ± 0.0	1.7 ± 0.3 *	3.0 ± 0.3 *	3.4 ± 0.5 *	3.0 ± 0.4 *	2.4 ± 0.4 *	1.7 ± 0.3 *	1.5 ± 0.2
	Control	1.0 ± 0.0	1.1 ± 0.1	1.3 ± 0.2	1.0 ± 0.0	1.1 ± 0.1	1.0 ± 0.0	1.1 ± 0.1	1.0 ± 0.0
CIR	**Exercise**	25.3 ± 0.6	25.6 ± 0.5*	25.8 ± 0.6*	25.9 ± 0.6*	25.8 ± 0.6*	26.1 ± 0.5*	26.1 ± 0.6*	25.9 ± 0.6*
(cm)	**Control**	26.3 ± 0.5	26.5 ± 0.5	26.4 ± 0.5	26.3 ± 0.5	26.4 ± 0.5	26.4 ± 0.5	26.4 ± 0.5	26.3 ± 0.5
ROM	**Exercise**	156 ± 2.9	147 ± 4.1 *	147 ± 3.7 *	148 ± 4.1	149 ± 3.6 *	151 ± 3.4	152 ±3.1	153 ± 3.2 *
(degree)	**Control**	152 ± 1.8	150 ± 2.2	150 ± 2.4	154 ± 1.9	154 ± 2.2	152 ± 2.3	153 ± 1.6	153 ± 1.9
CK ($U \cdot L^{-1}$)		142 ± 20	168 ± 24 *		500 ± 120*		817 ± 227*		800 ± 258*

Note: SOR refers to each subject's perception of muscle soreness. MVT refers to maximal voluntary isometric torque. ROM refers to the elbow's range of motion. CIR refers to arm's circumference at 8-cm above elbow. CK refers to plasma Creatine Kinase activity. * denotes significant ($p<0.05$) differences between day 0 Pre-EE and other days. Data are mean ± SE.

SOR, CIR and ROM: SOR and CIR increased and ROM decreased, significantly after EE, and these changes were persistent for the following 6-days (Table 1). The decrease

of MVT and onset of post-exercise DOMS symptoms, supported by biochemical changes within muscles that had undergone EE, was taken as prima facie evidence of muscle damage.

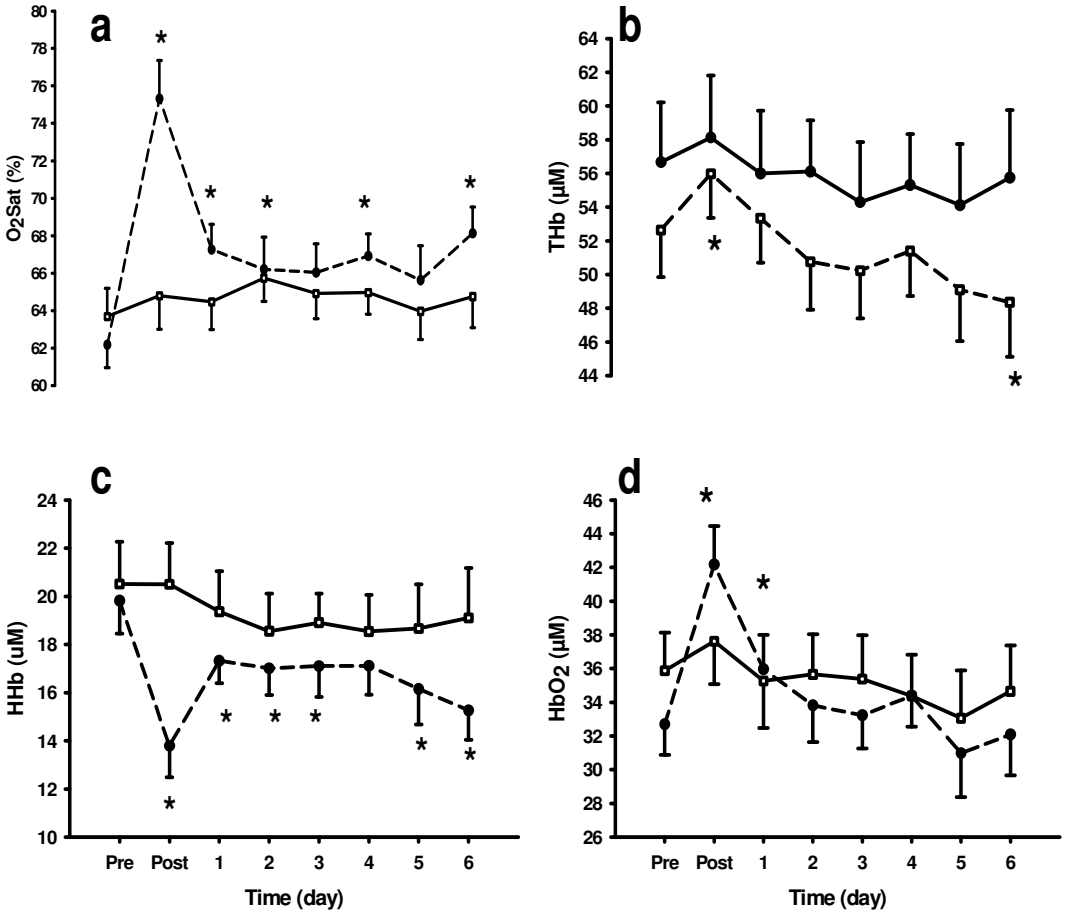

Figure 2. Resting (a) tissue oxygen saturation (O_2Sat), (b) total haemoglobin (THb) (c) deoxy haemoglobin (HHb) and (d) oxyhaemoglobin (HbO_2) for Control (solid line) and Exercise (dashed line) limbs. Control refers to non-EE arm and Exercise refers to EE arm. * denotes significant ($p<0.05$) differences between day 0 Pre-EE and other days.

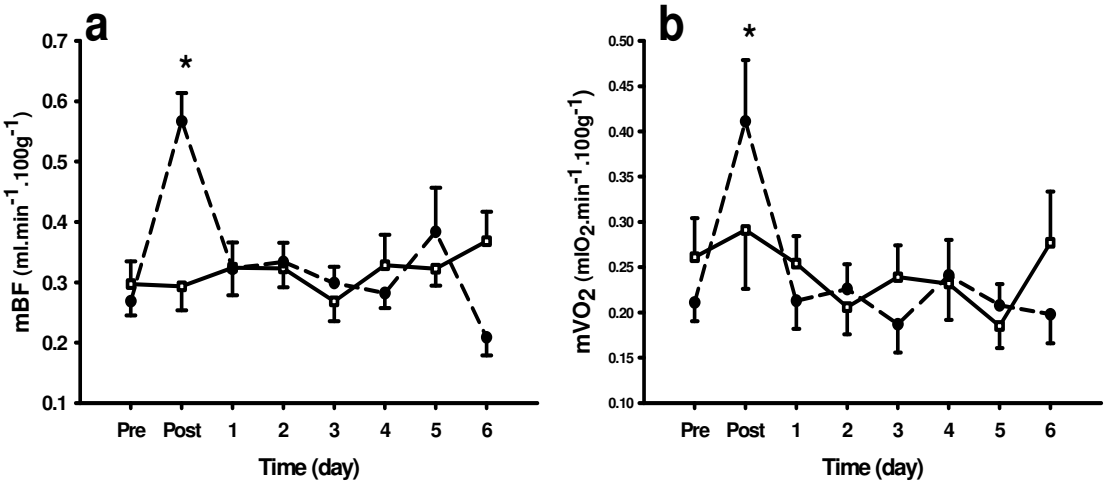

Figure 3. Resting (a) muscle blood flow (mBF) and (b) muscle oxygen uptake (mVO_2) for Control (solid line) and Exercise (dashed line) limbs. Control refers to non-EE arm and Exercise refers to EE arm. * denotes significant ($p<0.05$) differences between day 0 Pre-EE and other days.

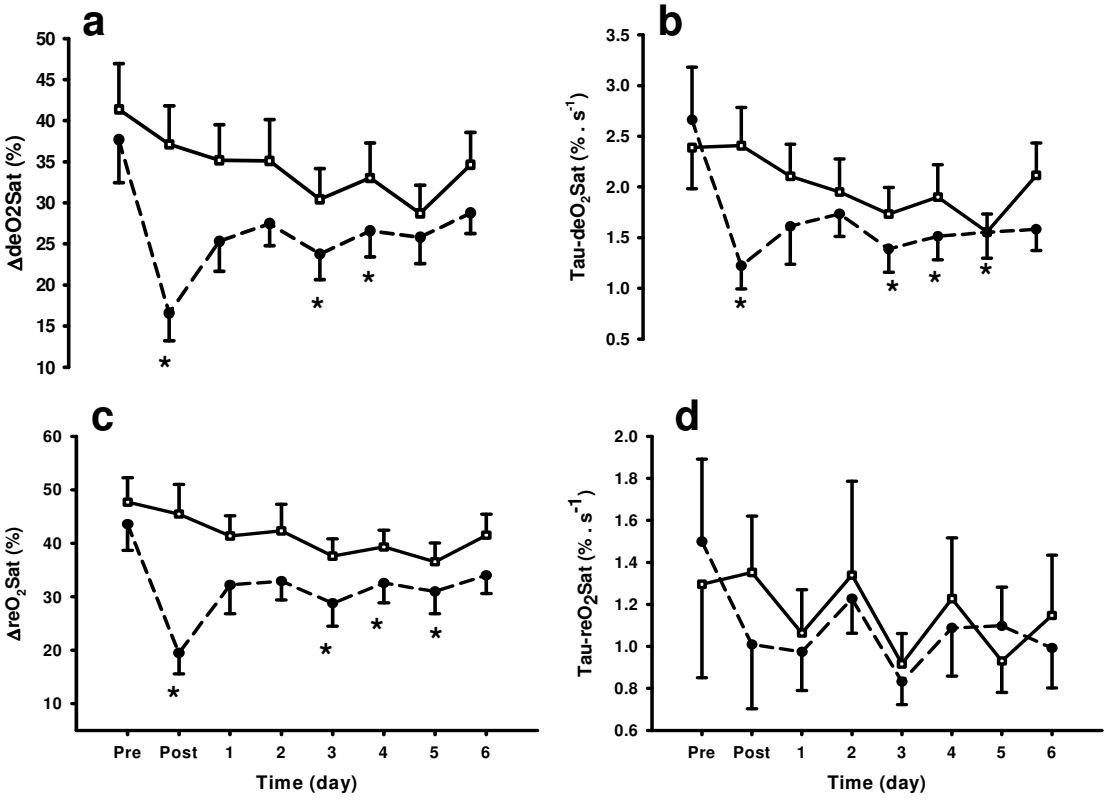

Figure 4. (a) ΔdeO_2Sat, (b) Tau-deO_2Sat, (c) ΔreO_2Sat, and (d) Tau-reO_2Sat for Control (solid line) and Exercise (dashed line) limbs during isometric contractions at 50% maximum voluntary contraction. Control refers to non-EE arm and Exercise refers to EE arm. * denotes significant ($p<0.05$) differences between day 0 pre-EE and other days.

NIRS-derived variables at rest: O_2Sat increased after acute EE and remained significantly higher than pre-EE values on days 1, 2, 4 and 6 (Figure 2-a). THb also increased after EE but recovered on day 1, and although it marginally decreased within the next 5-days, this decrease was only significant on day 6 (Figure 2-b). HbO_2 increased significantly after EE and did not recover until day 2 (Figure 2-d). In contrast, HHb decreased significantly after acute EE and remained lower than the pre-EE even by day 6 (Figure 2-c). Both mBF and mVO_2 increased significantly after acute EE, but recovered on day 1 (Figure 3-a and 3-b, respectively).

NIRS-derived variables during isometric contractions: ΔdeO_2Sat at IC50 decreased after EE and was significantly lower than pre-EE values on days 3 and 4 (Figure 4-a). Similarly, Tau-deO_2Sat and ΔreO_2Sat at IC50 (Figure 4-b and 4-c, respectively) decreased significantly after EE and remained significantly less than pre-EE values on days 3, 4, and 5. Tau-reO_2Sat did not change during IC50 (Figure 4-d). During IC80, ΔdeO_2Sat decreased after EE and was still significantly lower than pre-EE on day 1 (Figure 5-a). Additionally, Tau-deO_2Sat and ΔreO_2Sat at IC80 decreased significantly after EE and were lower than pre-EE values several days after EE (Figure 5-b and 5-c). No significant change was observed for Tau-reO_2Sat during IC80 (Figure 5-d).

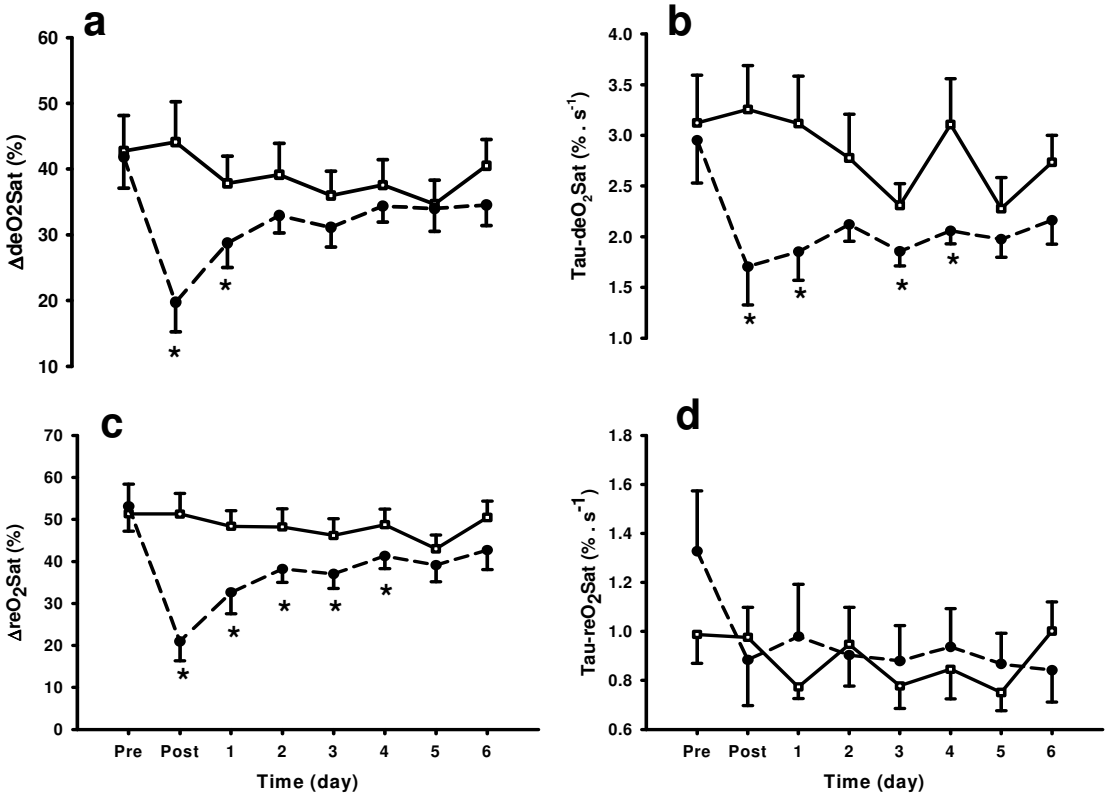

Figure 5. (a) ΔdeO_2Sat, (b) Tau-deO_2Sat, (c) ΔreO_2Sat, and (d) Tau-reO_2Sat for Control (solid line) and Exercise (dashed line) limbs during isometric contractions at 80% maximum voluntary contraction. Control refers to non-EE arm and Exercise refers to EE arm. * denotes significant ($p<0.05$) differences between day 0 pre-EE and other days.

Skinfold thickness correlation with NIRS-measured variables: No significant correlations were observed between skinfold thickness and resting O_2Sat ($r = 0.22$), mVO_2 ($r = 0.05$) or mBF ($r = -0.26$) for the Exercise arm. Similarly, no significant correlations were found between skinfold thickness and resting O_2Sat ($r = 0.29$), mVO_2 ($r = 0.36$) or mBF ($r = 0.12$) for the Control limb.

Discussion

The eccentric exercise protocol deployed in this study was modified from the previous studies that had induced a prolonged impairment of muscle function (Clarkson et al. 1992; Felici et al. 1997), and our findings are consistent with the results of those prior

studies. Prolonged loss of muscle force after EE is one of the most valid and reliable indirect measures of muscle damage in humans (Warren et al. 1999). In our study, a 30% to 60% decrease in MVT, that presented for 6-days after a single bout of EE, established prima facie evidence that the EE protocol had elicited muscle damage. Supporting the post-EE loss of muscle force and raised venous CK, changes such as significantly increased SOR and CIR, and decreased ROM were supporting evidence of EE's effectiveness to evoke damage.

Depending on the work intensity, the degree of activation of a particular muscle group and its trained state, skeletal muscles deoxygenate to varying degrees (Hampson and Piantadosi 1988; Boushel et al. 1998; Nioka et al. 1998). In addition, blood flow and O_2 delivery to a muscle will also affect its deoxygenation capability. The results of this study demonstrated mean O_2Sat increased by 22% after acute EE, and remained elevated by 5-9% for the following 6-days. In contrast, HHb values decreased by 30% after acute EE and remained depressed by 12%-23% over the next 6-days. Taken together, these changes suggested a general trend towards maintaining a higher percentage of available HbO_2 after exercise-induced muscle damage.

Increased energy expenditures usually require rapid adjustments of blood flow that affect the entire cardiovascular system. During exercise, the vascular portion of active muscles is considerably increased by the dilation of local arterioles (McArdle et al. 1986). This may serve to divert blood flow to areas of high demand such as exercising muscles. Muscle blood flow increased significantly after acute EE and this could explain the increases in O_2Sat, THb and HbO_2 that were observed immediately after EE.

Although there is not a certain explanation for the prolonged and significant alteration of muscle oxygenation and haemoglobin-associated measures, two possible mechanisms might be implicated. First, this could be a reflection of some inflammatory changes induced by EE, resulting in increased blood redistribution to the affected limb. Some authors (e.g. Nosaka and Clarkson 1996 44) have demonstrated the occurrence of muscle oedema after eccentric exercise, which could be due to the inability of the blood

vessels in maintaining blood outflow (Heap et al. 2006) on one hand, and increased blood flow to the damaged tissue on the other hand. We observed significant increases in arm circumferences of our subjects that may indicate the presence of oedema and inflammation. However, this study did not demonstrate a prolonged increase in mBF. Second, muscle damage results in increased mithochondrial Ca^{2+} concentration (Duan et al 1990) and therefore impairs muscle oxygenation. A reduced oxygen extraction due to mitochondrial disruption may result in a lower consumption of oxygen by the muscle fibre. A lower oxygen consumption might be reflected as a higher O_2Sat at the region of the NIRS probe.

Our NIRS data obtained during both IC50 and IC80 showed significant reductions over post-EE days for all NIRS-derived variables (except for Tau-reO_2Sat). These changes suggested that during brief isometric contractions, the exercised muscles became desaturated more slowly and to a lesser degree than those in the Control arm. This may suggest a shift towards anaerobic metabolism within the Exercise limb, possibly due to mitochondrial disturbances, increases in free radicals or other cellular disruption resulting from EE. Gleeson and colleagues (Gleeson et al. 1998) observed that venous blood lactate was higher in exercised group during incremental cycling two days after a session of bench stepping exercise (involving EE). They proposed that this might be due to an increase in the contribution of anaerobic metabolism for ATP production (Gleeson et al. 1998).

Newcomer and co-workers (Newcomer et al. 2005) have observed reductions in muscular oxidative capacity after bouts of acute unaccustomed exercise, accompanied by increased rates of anaerobic ATP production, fatigue, and delayed onset muscle soreness. Kano et al (2005) observed some microvascular disturbances after downhill eccentric running in rats. The authors noted that the observed changes may slow the exercise hyperaemic response at the onset of exercise and reduce the microvascular oxygen pressure available to drive blood-muscle O_2 delivery (Kano et al. 2005). In other words, their results suggested that one consequence of the microcirculatory disturbance after eccentric exercise could be slowed VO_2 kinetics, which might be associated with reduced contractile function and impaired exercise tolerance (Kano et al. 2005).

Furthermore, some intramuscular enzymatic process(es) are considered to set the speed of oxygen consumption kinetics in healthy individuals (Kano et al. 2005). These enzymatic process(es) could, thereby, establish the size of any O_2 deficit and the resulting intracellular perturbation (e.g., hydrogen ions, phosphocreatine, etc) upon initiating muscle contractions (Kano et al. 2005). Although we did not characterize enzymatic activity within the current study, the EE-induced muscle damage might have affected those processes.

Overall, the findings of our study supported the hypothesis that a session of unaccustomed eccentric contractions of elbow flexors might alter/impair muscle oxygenation and mBF within biceps brachii. These results supported the observations of Kano and colleagues (2004, 2005), although these prior studies were conducted on animals and used different techniques to assess muscle oxygenation and microcirculation. On the other hand, our findings were not in accordance with the results of Walsh and co-workers (Walsh et al. 2001), which to our knowledge is the only published data on muscle oxidative metabolism after EE derived from NIRS. Their group observed that eccentric cycling, although resulting in pronounced DOMS, was not associated with impaired oxidative function within muscle. The disparity between the results of the current study and those of Walsh and co-workers (Walsh et al. 2001) could be due to the exercise protocols each study used to induce muscle damage. There is more limb musculature involved in leg cycling exercise compared to eccentric extension exercise of the upper arm muscles that mainly reflect unilateral biceps brachii activation. The training status of subjects could be another factor to explain the disparity of findings. Walsh et al (Walsh et al. 2001) recruited Physical Education students for their study, while our subjects, while healthy, were relatively sedentary for 12-months prior to undertaking the EE stressor. Hence, a larger extent of damage or soreness was expected, and this was observed in our study. Further supporting this viewpoint, Walsh et al (Walsh et al. 2001) did not observe any significant changes in CK activity during their experimental period, which might suggest a lesser degree of muscle damage in their study, although it must be noted that CK activity alone is not a standard measure for muscle damage or soreness.

Additionally, our results were not fully in accord with the findings of Sbriccoli et al. (2001) and Laaksonen et al. (2006) who detected a marked increase in local blood flow for 2-4 days after EE. Although we observed a marked increase in mBF after acute EE, the alterations for days afterwards in mBF presented only a non-significant trend (P= 0.077). These differences in mBF findings amongst studies that have employed EE might reflect different blood flow measurement techniques. For instance, Sbriccoli and colleagues (2001) employed Doppler ultrasonography (which targets a larger area of blood supply) to quantify mBF, while we employed NIRS, which estimates mBF only in the region of the device's emitter-detector probe. It is worthy to note that a more precise measurement of mBF would be possible by the NIRS-monitoring of an injected tracer such as indocyanine green into the circulation (Boushel et al. 2000). Alternatively, our subject sample size and/or a lack of statistical power to detect a NIRS-derived mBF changes for days after EE might be other explanations.

Although adipose tissue thickness might have a confounding influence on in vivo NIRS measurements (Van Beekvelt et al. 2001), we did not observe a significant correlation between limbs skinfold thickness and resting O_2Sat, mVO_2 or mBF in this study. Maikala and Bhambhani (2006) have recently reported similar findings. However, it should be noted that in the current study, we measured skinfold adiposity only on day 0 prior to exercise and no correlation analyses could be performed on the subsequent days. Besides, our subjects were predominantly non-obese individuals (mean ± SD of their skinfold thickness were 4.84 ± 1.06 cm and 4.78 ± 0.79 cm for Control and Exercise arm, respectively), and although this reduces the chance of our NIRS-derived variables being affected by adipose tissue, it might have affected our correlation analyses. Changes in upper limb adiposity during the study period might have also had a confounding effect on our results.

Conclusion

The current study revealed a significant increase in resting oxygen saturation, decrease in deoxyhaemoglobin and similar changes in a few other parameters of muscle

oxygenation kinetics at rest and during exercise after strenuous EE. These changes might be due to a decrease in oxygen consumption resulting from mitochondrial disturbances, an increase in oxygen delivery due to increments in muscle blood flow, and/or a combination of both. As this study was the first to demonstrate such findings, further investigation using NIRS or NIRS in conjunction with other methods is warranted to identify the underlying mechanisms supporting to assess the changes in muscle oxygenation kinetics after eccentric exercise, and to elucidate whether these are affected by or affecting muscle damage.

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Chapter 4

Electromyographic Activity of the Biceps Brachii after Exercise-Induced Muscle Damage

As co-authors of the paper "Ahmadi S, Sinclair PJ, Foroughi N, Davis GM. Electromygraphic activity of the biceps brachii after exercise-induced muscle damage. *J Sport Sci Med*: 6(4), 461-70.", we confirm that Sirous Ahmadi has made the following contributions:

- conception and design of the research
- data collection
- analysis and interpretation of the findings
- writing the paper and critical appraisal of content

PJ Sinclair…..……...……...……Date:……

N Foroughi ……..Date:……

GM Davis …….……..Date:……...

Abstract

It is well known that strenuous eccentric exercise may result in muscle damage. We proposed that vigorous eccentric exercise (EE) would impair myoelectric activity of the biceps brachii. This study utilised a 7-day prospective time-series design. Ten healthy males performed a session of 70 maximal EE elbow flexion contractions. Analysis of surface electromyography activity (sEMG) was performed on the signals recorded during isometric contractions at 50% (IC50) and 80% (IC80) of maximum voluntary isometric torque (MVT), deriving RMS and MDF as sEMG parameters. Linear regression of the RMS and MDF time-series (20-s sustained IC50 and IC80) was used to extract intercepts and slopes of these signals on each day. Plasma creatine kinase activity (CK), MVT, arm circumference, subjective perception of soreness and elbow joint range of motion were also measured to assess effectiveness of EE to evoke muscle damage. CK increased over resting values until day 5 after EE, and remained significantly ($p<0.05$) elevated even on day 7. MVT had decreased to 45% of its initial value by day 2 after EE, and remained significantly depressed for the following 6 days. In addition, muscle soreness and arm circumference increased, and range of motion decreased after EE. A significant shift of MDF intercept towards lower frequencies at both IC50 and IC80 was observed after EE in the exercised arm, and these values gradually recovered within the next 3 days during IC50. Although there were some changes in RMS values, these alterations were persistent in both control and exercised arms, and did not follow a consistent pattern. In conclusion, a prolonged reduction in MDF intercept was observed after EE, but this was not closely time-associated with the biochemical, anthropometric or functional markers of muscle damage. Compared to RMS, MDF was a more consistent measure to reflect changes in sEMG.

Key Words: Eccentric Exercise, Creatine Kinase, Surface Electromyography, Median Frequency, Root Mean Square.

Introduction

Eccentric exercise (EE) generates greater tension per active muscle fibre than concentric or isometric contractions, resulting in mechanical disruption of the muscle fibre (Clarkson and Sayers 1999; Lieber and Friden 2002). Delayed onset muscle soreness and impaired muscle function are the common consequences of excessive EE. Impaired glycogen resynthesis (O'Reilly et al. 1987), myofibrillar damage along the Z-band (Clarkson and Hubal 2002), mitochondrial swelling and increased intramuscular pressures (Friden et al. 1983) are some of morphologic and metabolic signs of muscle alteration post-EE that are associated with muscle damage.

Exercise-induced muscle damage have been evaluated both directly (Stauber et al. 1990; Miura et al. 2000) and indirectly (Sayers et al. 2001; Chen 2003). However, due to the invasive nature of direct studies, such as muscle biopsy, indirect methods of evaluating muscle damage have been preferred, and these have generally been utilized in human studies. Some examples of indirect methods have included measuring changes in plasma creatine kinase (CK) activity, perceived muscle soreness (SOR), maximum voluntary torque (MVT), inflammatory markers (in plasma and muscles), neuromuscular function (measured by electromyography, EMG), MRI signal intensity, and muscle oxygenation and blood flow (measured by ultrasound, plethysmography and near infrared spectroscopy) (For a review see Clarkson and Hubal 2002). However, the accuracy and reproducibility of some of these muscle damage assessment techniques are somewhat uncertain, and further improvements and investigations on the application of these techniques are warranted.

Surface electromyography (EMG) is a technique for evaluating and recording physiologic properties of muscles at rest and during exercise. Electromyograms monitor and record neuromuscular action potentials as myoelectric signals. Two fundamental types of variables can represent EMG: frequency and amplitude. The underlying physiological processes associated with the excitation of motor units dictate the constituent frequencies that produce the generated myoelectric signal. Therefore, the frequency content of the recorded signal can be related to the numbers of units active as well as their constituent firing rates (Kamen and Caldwell, 1996). One popular measure

of EMG frequency content is median frequency (MDF), the point at which the spectral power is divided into equal low- and high-frequency halves. MDF is a recomonded variable for the study of muscle fatigue and damage (Merletti et al., 1995, Felici et al., 1997). On the other hand, EMG amplitude, which is usually presented as root mean square (RMS) of the signals, can also provide information about number and location of active motor units, recruitment of motor units and shape of motor unit action potentials (Felici et al. 1997).

The analysis of EMG has been used to detect changes in the contractile properties of a muscle both during and after EE (Berry et al. 1990; Felici et al. 1997; McHugh et al. 2000). However, there are disagreements amongst different researchers who have studied the effect(s) of EE on EMG signals. For instance, Komi and Viitasalo (1977) and Berry and colleagues (1990) observed some increases in EMG activity after EE, while Day et al (1998) did not find any significant change in this parameter. Similarly, Day and co-workers (1998) and Felici et al (1997) observed significant decreases in mean and median frequency, while Berry and colleagues (1990) did not observe any consistent change in EMG frequency after EE. The reason for such divergence of findings is unknown, although they could be partially attributed to methodological differences in inducing muscle damage (and therefore the magnitude of morphological disruption) or to different methods of analysing data amongst previous studies. However, this issue needs to be further investigated to determine; (i) whether EE results in any changes of neuromuscular activity within exercised muscle, (ii) if there are any changes, what might be the possible mechanisms underlying these, and, (iii) can EMG be used as a tool to assess exercise induced-muscle damage. Therefore, in this study, the effects of EE on some of the physiological characteristics of the muscle assessed via surface electromyography were investigated. We hypothesized that MDF and RMS will shift to lower and higher values, respectively. These changes in MDF and RMS could be primarily due to the possible impairments in the function of fast-twitch fibres resulting from unaccustomed eccentric contractions.

Methods

Subjects: Ten healthy males (age 25.4 ± 4.3 yr, body mass 73.1 ± 9.8 kg, stature 173.2 ± 7.0 cm; mean ± SD), who had not participated in any regular upper body muscular exercise training (i.e. at least 2-times per week for more than 30-mins) for 12-months prior to commencing the study, took part in the experiment. The Human Research Ethics Committee of the University of Sydney approved this study. All subjects were informed of the purpose, nature, and potential risks of the investigation, and gave their written informed consent to participate. Subjects were in healthy physical condition with no signs or symptoms of neuromuscular disease. They were not under pharmacological treatments and followed a normal diet. All participants were requested to abstain from any exercise involving arm muscles for the duration of the study.

Study design: The exercise protocol used in this investigation was modified from previous studies that had been designed to induce muscle damage (Clarkson et al. 1992; Felici et al. 1997; Sbriccoli et al. 2001). Subjects were habituated to the equipment and performed some isometric contractions similar to those that they would perform during testing days. Testing sessions were performed over a 7-day period. Each subject's biceps brachii of the non-dominant arm performed EE (Exercise), whereas the biceps brachii of the dominant arm was employed as non-exercise limb (Control).

Eccentric exercise protocol: On the first day, subjects performed two sets of 35 maximal voluntary EE contractions (5-s of contraction and 12-s of passive recovery) with the elbow of their non-dominant arm placed in an isokinetic strength machine. Subjects were requested and encouraged both verbally and visually (force output on a computer monitor), to maximally resist elbow extension movements in which the arm was forcibly extended from an elbow-flexed (on average 50°) to an elbow-extended (on average 170°) position at a preset angular velocity of 45 $deg \cdot s^{-1}$. The apparatus brought subject's arm back to the elbow-flexed position after each eccentric contraction. The two EE sets were separated by a 5-min recovery interval.

Resting assessments

Before and 30-min after the EE session on day 1 and for the next 6-days, the following measurements were made on Exercise and Control arms. It should be noted that Creatine Kinase (CK) activity were not assessed in all sessions for methodological reasons of minimising repeated phlebotomy, however the order of performing the measurements was the same within each session.

Plasma CK activity (CK): CK was measured on day 1 before and after EE, and on days 3, 5, and 7 at the beginning of each isometric exercise session. At each sampling time, about 5-ml of venous blood was withdrawn from the antecubital vein, centrifuged for 10-min to extract plasma samples, and analysed for CK activity within 24-hr. Plasma CK activity was assayed spectrophotometrically at 37°C using CK-NAC reagent kits (Thermo electron CORP., USA). Each plasma sample was assayed at least twice, until two assays were within 10% of the lower value and the mean of the two values was used for statistical analyses.

Elbow range of motion (ROM): Subjects were instructed to stand beside a whiteboard in a relaxed position with their investigated arm relaxed (extended position). At this time, an experienced investigator marked the locations of shoulder (Acromion), elbows (Olecranon) and wrist (Styloid process) on the whiteboard, and measured the resultant angles using a goniometer. The subject then flexed his forearm while the elbow and shoulder joints were kept constant with the assistance of another investigator. The new position of wrist was marked again on the whiteboard (flexed position) and the angle was measured. The difference between extended and flexed positions was taken into account as ROM. This was repeated 3-times and the average of the 3 ROM was used for statistical analyses.

Arm circumference (CIR): CIR was measured at 4, 6, 8, and 10-cm above the elbow joint, while allowing the arm to hang down by the side. The average of the three trials for CIR was used for statistical analyses.

Perception of muscle soreness (SOR): A subjective rating of SOR was performed during each session using a 7-point categorical scale, where 1 corresponded to "no pain" and 7 to "very, very painful". While standing, the subjects were instructed to palpate their upper arm during full range of motion biceps curls and then choose the number that corresponded to their perceived level of soreness (Sayers et al. 2000).

Exercise assessments

After subject placement, equipment set up and resting measurements (described above) were effected, the following exercise assessments were performed.

Isometric contractions: Isometric maximal voluntary contraction (MVT) was assessed on the Exercise and Control limbs with the subject's elbow joint set to 90° and shoulder flexed at 45° using an isokinetic strength-assessment apparatus (Biodex System 2, Biodex, USA). Three 5-s repetitions were performed with 2-min of recovery between each maximal effort. The highest value was taken to represent the 100% MVT, and was employed for statistical analyses.

After a further 5-min of recovery, isometric elbow flexions were performed on the Exercise and Control limbs at 50% of MVT (IC50). The highest MVT from the first day before EE was used to set IC50 for all subsequent sessions. The rationale for the IC50 test was to set a constant muscle contraction force for all days before and after the EE stimulus. The 50% level was chosen after pilot testing to achieve a force that all subjects would be able to achieve after EE. During each session, subjects performed two isometric contractions at IC50, each lasting 20-s with 3-min of recovery between efforts.

Isometric elbow flexions were also assessed on the Exercise and Control arms at 80% of the MVT recorded for that particular session (IC80). The torque values for IC80, therefore, varied amongst days, depending on the MVT achieved for that day. Our rationale for the IC80 test was to assess EMG at a consistent level of effort before and after eccentric exercise-evoking muscle damage. Two contractions were performed at

IC80 of equal duration and recovery intervals as for IC50. Participants could observe their effort to reach and maintain the average exercise intensity requested by the investigator (50%, 80%, or 100% of MVT).

IC50 and IC80 assessments were performed every day. On the first day only, an average of 10-min after the last IC80, subjects performed EE contractions (as described in 'EE protocol'). Then, subjects were rested in a comfortable position for 30-min and the pre-EE assessments repeated. The experimental protocol measured one arm at a time and subjects were unaware which was going to be assessed first. However, on day one after the pre-EE assessments on Control and Exercise limbs, subjects performed eccentric contractions with their Exercise arm, then after 30-min passive rest, their Exercise and Control arms were assessed, respectively (post-EE assessments). The experimental sessions were set at the same period every day (in the mornings) for each subject, and room temperature was set between 23°C to 25°C for all subjects.

Electromyography: EMG signals were recorded from the biceps brachii muscle. The skin was prepared by shaving, abrading, and cleaning the recording area with alcohol. Bipolar electrodes (9-mm, square shape, Tyco Health Care, H49P Cloth Solid Gel ELEC C450) were fastened over the belly of the biceps brachii muscle, parallel to fibres, with a centre to centre distance of 35-mm. A passive reference electrode was placed on the dorsal surface of the wrist. Electrolyte gel was used to improve signal conduction between the skin and the electrodes. The electrode positions were selected and marked with a semi-permanent marker to assure standardized measurements from day to day. Electrode placement was preceded by abrasion of the skin to reduce the source impedance to <5 kΩ.

EMG was recorded using a Medelec Amplifier (MS6, input impedance of 500-MΩ// 30-pf, common mode rejection ratio of greater than 10,000:1 at 50-Hz). The raw signal was filtered (10-Hz to 1-kHz), monitored on a digital oscilloscope and digitized at 1000 Hz using a 12-bit analogue-to-digital (A/D) converter on a computer. Gain was adjusted to maximize resolution.

During each 20-s isometric contraction, the participants were asked to reach the required percentage of MVT in less than 2-s and maintain it for 18-s using real-time feedback displayed on a computer monitor. RMS and MDF are the two EMG-derived variables that have been used frequently in previous EMG studies (Felici et al. 1997; Linnamo et al. 2000; Hermann and Barnes 2001; McHugh et al. 2001). Using purpose-built software (Bioproc2, Robinson G., University of Ottawa, Canada), RMS and MDF values were extracted at time epochs of 1-s.

Statistical analysis

For the EMG data, linear regression analyses of the time course of RMS and MDF (RMS and MDF against 1-s time epochs) were performed on the collected data (Merletti et al. 1990; Felici et al. 1997). To omit on- and off-transient phenomena associated with muscular exertion, the first and last 2-s of every contraction were discarded; therefore for each trial, there were 16-s of isometric effort (Figure 1). The axis intercepts ($\beta 0$) and slopes ($\beta 1$) of regression lines for RMS and MDF were used for statistical analyses. Prior to statistical analyses of RMS and MDF intercepts and slopes, a Chi-Square established that these were significantly different from cipher (0). Axis intercepts were employed as an index of muscle activation, while slopes represented the rate of fatigue in exercising muscles. The axis intercepts were assumed to be the indicative initial state of muscle activation, but because the first 2-s of each contraction had been discarded, this might slightly underestimate the "true" intercept (Merletti et al. 1990).

A t-test was performed to test the null hypothesis of similarity of linear regression coefficients ($\beta 0$, $\beta 1$) for the 2 trials of IC50 and IC80 within each session. Figure 1 shows an example of such a test for two trials on the same arm. As there were no significant differences between trials, data from both trials were combined for subsequent analyses.

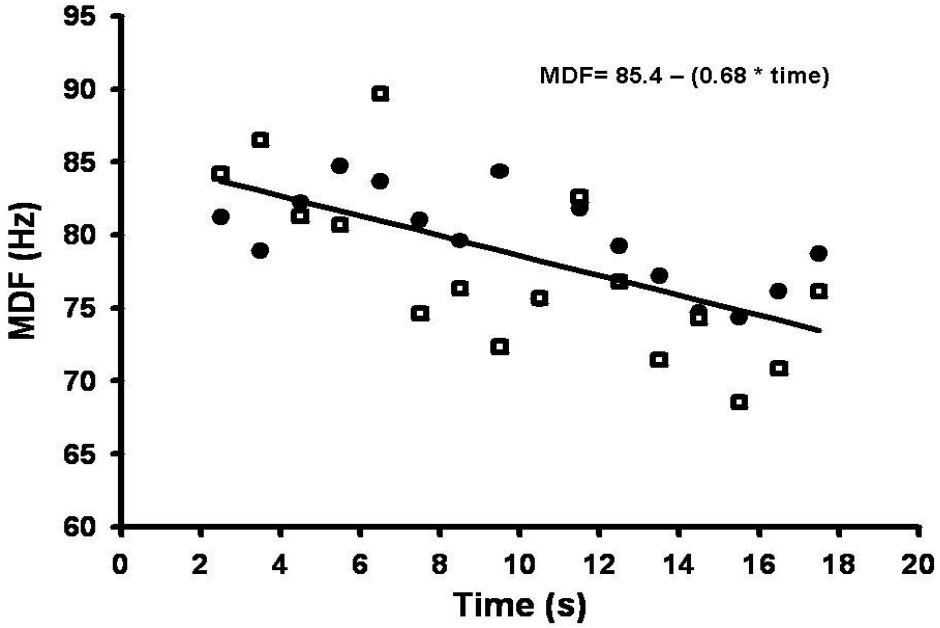

Figure 1. Sample plot of MDF vs. time during IC50 for Exercised arm before EE. The open (□) and filled (●) symbols denote the two muscle contractions performed. Also shown is the linear regression line for both contractions, combined.

A two-way analysis of variance (ANOVA) with repeated measures was used to test the main effect of arm (Control, Exercise) by time (day one to day seven) for all variables except for CK activity. When a significant arm-by-time interaction effect was observed, univariate ANOVA was performed for each arm. For CK, since blood was drawn only from one arm, a separate univariate ANOVA was performed without the arm main effect. We used SPSS (version 14) for the statistical analyses and statistical significance for a meaningful change was set at the 95% confidence level ($p < 0.05$). Values reported as mean ± the standard error of means (SE).

Results

Significant arm-by-day interaction effects were observed for MVT, ROM, CIR (at 6, 8 and 10 cm above elbow), and SOR (active, passive, flexed and extended). Therefore, further statistical analyses were performed on each arm separately, and these revealed

that in the Exercised arm, there was a significant decrease in MVT immediately after EE (Figure 2), that remained lower than initial values for the following 5 days. ROM also decreased after EE and gradually returned towards initial levels over the following 5 days (Table 1). CIR significantly increased at all four measurement locations on the day two, and over the following 6 days (Table 1). Similarly, SOR increased after EE, and remained higher than pre-EE over the next 5 days (Table 1). CK also increased significantly after EE, and it remained higher than the initial values for the following days (Figure 3).

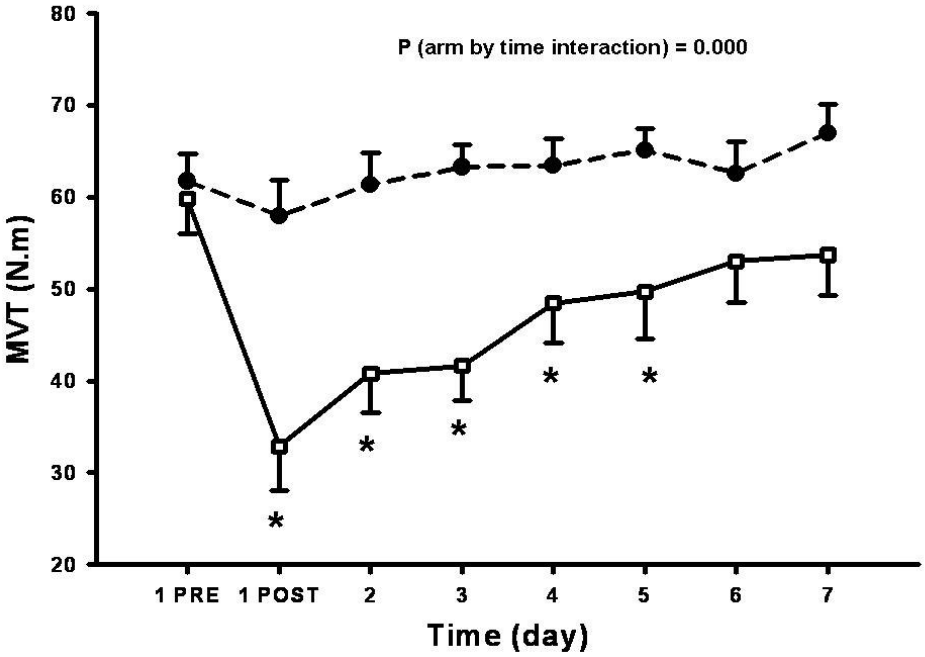

Figure 2. MVT before and after EE sessions for Control (●) and Exercised (□) arms. On the X-axis, 1 PRE refers to the first session (before EE) and 1 POST refers to the session immediately after EE on day 1. * denotes a value significantly different from Day 1 before EE (1-PRE), $p<0.05$. Data are mean ± SE.

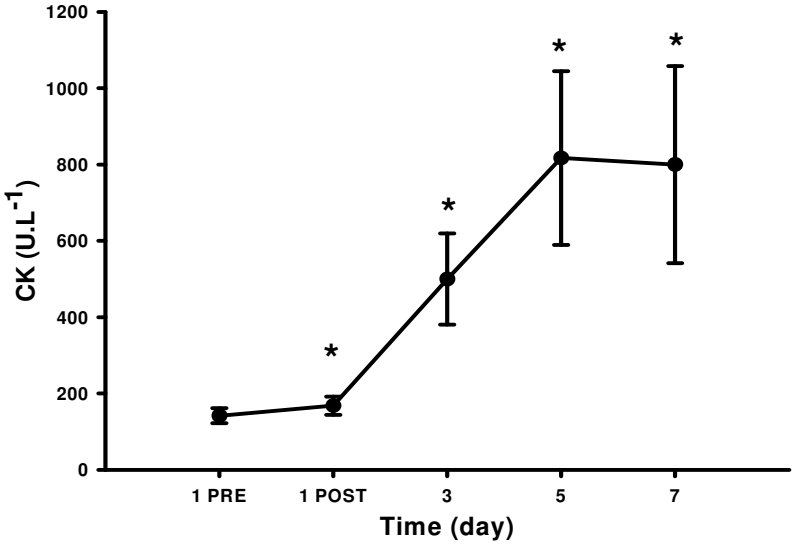

Figure 3. Blood-borne CK activity before and after EE session. On the X-axis, 1 PRE refers to the first session (before EE) and 1 POST refers to the session immediately after EE on day 1. * denotes a value significantly different from Day 1 before EE (1-PRE), $p<0.05$. Data are mean ± SE.

During IC50, MDF intercept (β_0) decreased significantly after acute EE, and was significantly lower than pre-EE values on day 3, but gradually returned towards the initial values over the next 2 days (Figure 4). No significant arm-by-day interaction effects were observed for MDF slope (Table 1), RMS intercept (Figure 5) and RMS slope (Table 1) during IC50.

Table 1. Summary of physical signs and EMG for the Exercised arm.

	DAY 1 PRE	*DAY 1 POST*	*DAY 2*	*DAY 3*	*DAY 4*	*DAY 5*	*DAY 6*	*DAY 7*
ROM (deg) †	156 ± 2.9	147 ± 4.1 *	147 ± 3.7 *	148 ± 4.1	149 ± 3.6 *	151 ± 3.4	152 ±3.1*	153 ± 3.2
CIR (cm) †	25.3 ± 0.6	25.6 ± 0.5	25.8 ± 0.6 *	25.9 ± 0.6 *	25.8 ± 0.6 *	26.1 ± 0.5 *	26.1 ± 0.6 *	25.9 ± 0.6 *
SOR †	1.0 ± 0.0	1.7 ± 0.3 *	3.0 ± 0.3 *	3.4 ± 0.5 *	3.0 ± 0.4 *	2.4 ± 0.4 *	1.7 ± 0.3 *	1.5 ± 0.2
MDF IC50 slope (β1; Hz• s^{-1})	-0.55 ± 0.06	-0.62 ± 0.08	-0.81 ± 0.01	-0.57 ± 0.08	-0.41 ± 0.11	-0.42 ± 0.08	-0.45 ± 0.08	-0.59 ± 0.04
MDF IC80 slope (β1; Hz• s^{-1})	-0.90 ± 0.16	-0.54± 0.09	-0.89± 0.10	-0.68± 0.15	-0.65± 0.11	-0.57± 0.09	-0.69 ± 0.10	-0.72 ± 0.11
RMS IC50 slope (β1)	0. 011 ± 0.005	0.013 ± 0.005	0.005 ± 0.004 *	0.002 ± 0.003 *	-0.003 ± 0.004	-0.001 ± 0.002 *	0.004 ± 0.002	0.002 ± 0.002 *
RMS IC80 slope (β1)	0.017 ± 0.009	0.004 ± 0.005	0.001 ± 0.002	0.008 ± 0.005	0.010 ± 0.004	0.004 ± 0.005	-0.001 ± 0.006	0.005 ± 0.004

Note: SOR refers to each subject's perception of muscle soreness while arm was actively extended. ROM refers to active elbow range of motion. CIR refers to arm circumference at 8-cm above elbow. CIR at all anthropometrical landmarks (e.g. 4, 6, and 10-cm above elbow) followed a similar pattern, therefore, data was only presented from CIR at 8-cm. MDF refers to EMG median frequency. Derivation of regression intercept (β0) and slope (β1) are described in the text. † indicates a significant interaction between main effects of arms × time (days); * denotes significantly ($p<0.05$) different from day 1 PRE-EE. Data are mean ± SE.

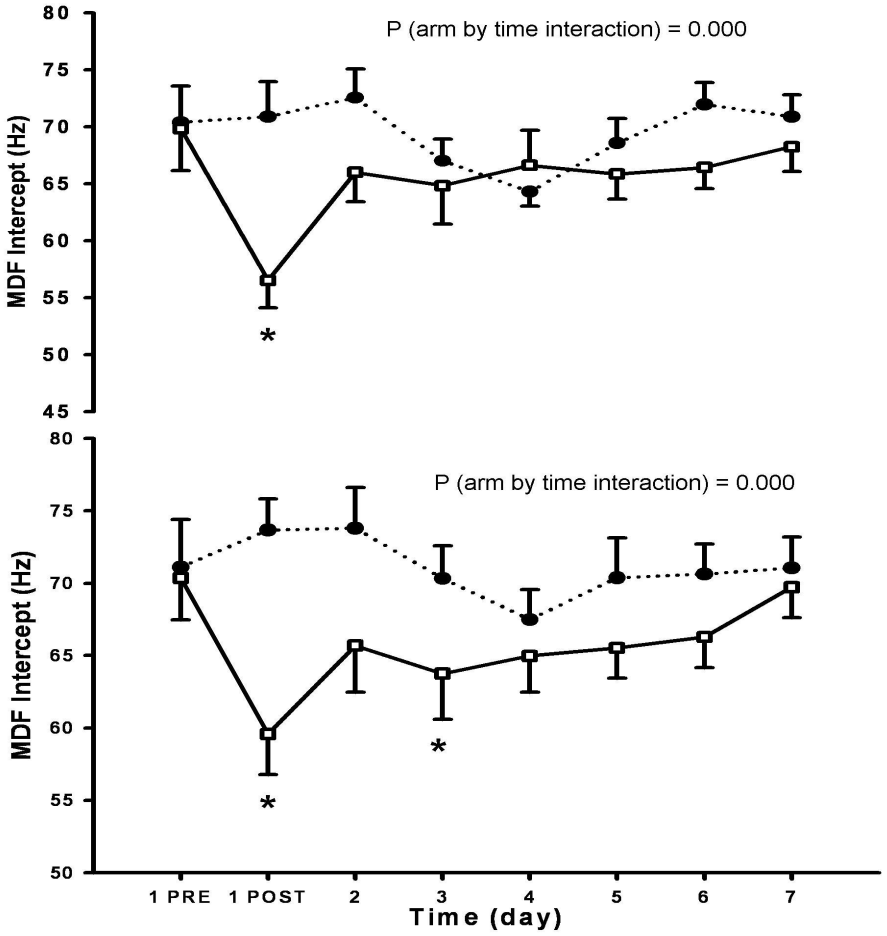

Figure 4. MDF intercept coefficient (β0) on different days at IC50 (lower panel) and IC80 (upper panel) for Control (●) and Exercised (□) arms, respectively. Derivation of β0 is described in the text. On the X-axis, 1 PRE refers to the first session (before EE) and 1 POST refers to the session immediately after EE on day 1. * denotes a value significantly different from Day 1 before EE (1-PRE), $p<0.05$. Data are mean ± SE.

There were significant arm-by-day interaction effect for MDF and RMS intercepts at IC80. Subsequently, a univariate ANOVA showed a significant decrease in MDF intercept after acute EE within the Exercised arm, which recovered afterwards (Figure 4). Although there was a significant arm-by-day interaction for RMS slope, the pattern of change was not consistent for either arm (Figure 5). No significant changes were observed for MDF and RMS slopes at IC80 (Table 1).

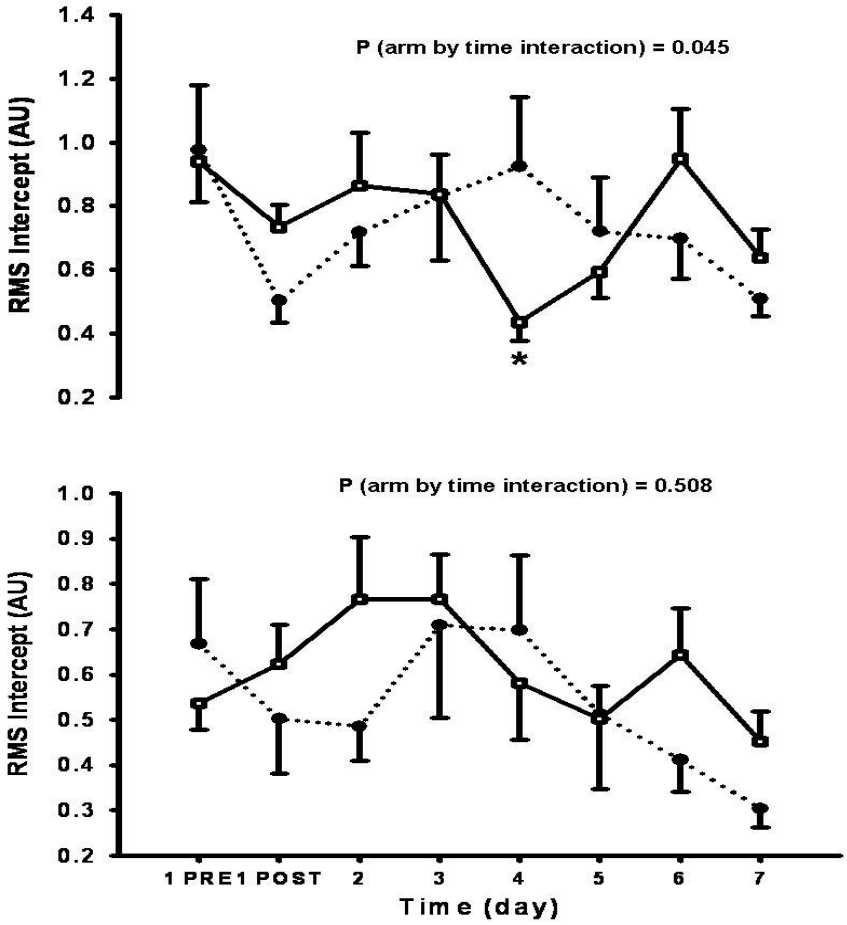

Figure 5. RMS intercept coefficient (β0) obtained on different days at IC50 (lower panel) and IC80 (upper panel) for Control (●) and Exercised (□) arms, respectively. Derivation of β0 is described in the text. On the X-axis, 1 PRE refers to the first session (before EE) and 1 POST refers to the session immediately after EE on day 1. * denotes a value significantly different from Day 1 before EE (1-PRE), $p<0.05$. Data are mean ± SE.

Discussion

The purpose of this study was to investigate the possible physiological changes within muscle assessed via surface electromyography after a session of heavy EE. The unique finding of this investigation was that EE-induced muscle damage revealed some significant alterations in surface EMG for up to seven days after exercise. Prolonged

and significant decreases in MVT and ROM, and increases in CIR, SOR and CK were consistent with exercise-induced muscle damage. However, it is worthy to note that our perception of muscle damage was based on indirect measures such as CK, MVT, or physical signs, which are not categorical methods of determining muscle damage (e.g. muscle needle biopsy). Therefore, we have employed the term "muscle damage" with some caution.

In the present study, to track EMG parameters obtained day-to-day over seven days after EE, subjects were asked to perform isometric contractions at 50% of their MVT measured on the very first session (e.g. day 1 before-EE). That is, subjects were required to apply the same absolute amount of force to perform IC50 on every day of the 7-day trial. However, to elicit a higher level of muscle fibre recruitment, subjects also performed isometric contractions at 80% of their MVT obtained on each particular day. Therefore, the amounts of force to perform IC80 were different between sessions depending on subjects' MVT during that session. We selected this approach to document possible changes of RMS and MDF at a constant level of force, as well as at a consistent level of effort. In this study, we did not normalize our EMG data based on daily EMG measures, because to do so would eliminate any changes that occurred between days. It is interesting to note that the 50% and 80% of MVT on days 2 and 3 often evoked similar isometric forces, because the subjects' maximum force declined significantly on those days. With the above approach in mind, when we aimed to compare the day-to-day changes in EMG, IC50 might be relevant to describing muscle physiological responses at the same absolute levels of torque production. But, when wishing to study responses of a constant level of effort, IC80 might provide the best way to document muscle physiological adaptations during post-EE recovery.

Although our findings showed significant arm-by-day interaction in RMS regression intercepts during IC80 (Figure 5), the overall changes did not follow a consistent trend or pattern. Similarly, we did not observe a consistent pattern of changes in RMS intercept and slope at IC50 or in RMS slope at IC80 (Table 1). Therefore, it seemed that RMS did not provide reliable information about muscle recovery after damage. RMS is more susceptible to the day-to-day changes compared to MDF (Merletti et al. 1995;

Felici et al. 1997). These results supported the findings of other authors who either did not find any significant changes in RMS after EE (Sayers et al. 2001), or their RMS data was not statistically linear, and they did not perform further statistical analyses on RMS (Felici et al. 1997). However, the lack of change in RMS intercept despite a 45% decrease in MVT may indicate that strength loss was primarily a function of mechanical disruption of the contractile component and not decreased ability to activate the muscle.

MDF linear regression intercept decreased significantly after acute EE during both IC50 and IC80 within the Exercised arm and was also less than pre-EE on day 3 during IC50 (Figure 4). Although MDF had recovered by day 2 during IC80, there was a general trend of decrements over the next 5 days for this variable. This was also the case for MDF intercept at IC50 (Figure 4). These findings were in accord with the findings of some studies (Felici et al. 1997; Day et al. 1998), but not in line with the results of others (Komi and Viitasalo 1977; Berry et al. 1990; McHugh et al. 2000). McHugh and co-workers (2000) reported that median frequency did not change after EE. Berry et al. (1990) did not observe any significant change in mean frequency after EE.

The possible reason for a decline in MDF after EE might be explained on a physiological basis. MDF represents information about conduction velocity of muscle fibres, the shape of motor unit action potentials, the mean firing rate of the individual motor units, the recruitment of motor units and the extent of superposition of action potentials from concurrently active motor units (Felici et al. 1997). Muscle fibre conduction velocity is higher for fast-twitch fibres (Andearssen and Arendt-Nielsen 1987), which means that when fast twitch fibres are more active the MDF value will be higher. Friden et al (1983) observed that fast twitch fibres showed significant disruption at the myofibrillar Z-band after EE compared to the other types of muscle fibres (Friden et al. 1983). Therefore, fast twitch fibres are more susceptible to damage and fatigue (Berry et al. 1990), and consequently a shift towards greater recruitment of slow twitch motor units might be anticipated in order to decrease the stress on the susceptible fast-twitch fibres (McHugh et al., 2001). Therefore, a decrease in MDF could be the result of a preferential reduction in the recruitment of fast-twitch fibres. On the other hand, the changes in intra muscular pressure, as well as the changes in water content and blood

volume of the muscle could have affected the EMG findings. Blood flow can affect characteristics of surface-recorded signals by imposing a low-pass filter medium. This tissue filtering can decrease the frequency content of the signal (Kamen and Caldwell, 1996). Additionally, an increase in blood flow generally increases local temperature, which can change spectral features of the EMG signals (Holewijn and Heus, 1992). Finally, another possible reason for a decline in MDF is a possible decrease of action potential firing rate produced by the motor cortex, which occurs during prolonged contractions (Bigland-Ritchie et al 1983).

Some of the reasons for the dissimilar outcomes of our study compared to previous investigations might include dissimilar methodologies and different muscle groups that were employed to study the EE-induce muscle damage. In addition, different methods have been used to quantify EMG activity. For example, McHugh et al. (2000) obtained their MDF data from an MVT test, while MDF in the current study was derived from isometric contractions at pre-set percentages of subjects' MVT. In voluntary exercise, e.g. where a MVT is performed, there is always some variation in the instantaneous force due to motivation and other factors. This may consequently increase the variance in EMG data and mask the effect of EE on EMG signal. Therefore, some changes in the EMG parameters during maximal contractions could be attributed to factors such as motivation (Linnamo et al. 2000). By obtaining the EMG data from IC50, which was a constant level of force based on the MVT of day 1, we minimized the effect of subject's motivation on EMG acquisition. The EMG power spectrum has been shown to be reliable for measurements during isometric contractions with a given intensity, repeated over separate days (Linnamo et al. 2000). Additionally, the magnitude of muscle damage might have been relatively less in McHugh et al. (2000) compared to our study. Although they did not assess CK, the percentage of decrease in muscle strength after EE in the McHugh and colleagues' (2000) study (10%) was less than ours (45%). A lower reduction in MVT (and probably a less magnitude of muscle damage) could be due to the lower level (60% of MVT) of EE intensity that they employed to induce muscle damage, compared to our study (on average 100% MVT). Further more, McHugh and colleagues (2000) assessed the myoelectric activity of hamstrings. Their subjects, therefore, sat on the EMG electrodes during the tests. Sitting on the electrodes during

hamstring contractions might have changed the orientation of the electrodes to the motor point of the respective muscles (McHugh et al. 2000)

One of the methodological differences that could be observed between our study and that of Berry and co-workers (1990), is that their group employed mean frequency, resulting from 10 subsequent samples. Averaging the mean frequency values possibly smoothed their results. Besides, Berry et al. (1990) performed their EMG assessments while their subjects lifting their own legs off the ground. Although, the same assessments were performed before and after EE, it is not clear that this leg-lifting was equivalent to any known percentage of MVT. However, one can assume that the leg – lifting exercise would require a force level of much lower than 50% of MVT. The myoelectrical behaviour of muscles could be different during low vs. high intensity contractions (Felici et al. 1997). In other words, the higher force produced during EMG acquisition in our study compared to that of Berry et al (1990), probably better revealed any physiological changes within the muscle.

In this study, we observed that MDF decreased over time during sustained isometric contractions (Figure 1). These decrements, which were shown as MDF slopes, were present in both Control and Exercised arms and at both intensities (IC50 and IC80). However, there were not any significant day-to-day changes amongst the slopes obtained from different arms and different intensities (Table 1). This suggests that in a sustained situation such as a 20-s isometric contraction, the rate of decrease in MDF, which could also be assumed as a rate of fatigue, was independent of EE-induced muscle damage. A possible mechanism for the decrease in MDF during prolonged contractions is the external accumulation of potassium ions (Mills and Edwards 1984). An outward leakage of potassium resulting in an ionic imbalance around sarcolemma might slow the action potential and consequently decrease MDF (Day et al. 1998).

Kroon and Naije (1991) observed a significant increase in the slope of mean power frequency immediately after EE, which recovered gradually within the consequent few days. Although our results followed a similar pattern to those of Kroon and Naije (1991), the changes in regression slope coefficient observed in our study were not

statistically significant (Table 1). The reason(s) for this disparity of findings is not clear. Kroon and Naije (1991) recruited five subjects, which is a relatively small group compared to our cohort (n=10). This might have induced larger inter-subject variations in EMG parameters. They did not delete any of the EMG data obtained during isometric contractions, while we deleted the first and the last 2-s to skip the transition phenomenon that could affect the EMG outcomes. Finally, their subjects performed different number of eccentric contractions at 40% of their MVT before they become exhausted, while in our study, the subjects performed a certain number of contractions (2 sets of 35) at100% MVT (on average). A lower EE intensity could, therefore, result in a lesser magnitude of muscle damage, and this might have affected the fibre recruitment and consequently the EMG signals after EE.

Although, our findings showed significant arm-by-day interactions for RMS regression intercepts during IC80 (Figure 5), the overall changes did not follow a consistent trend or pattern. Similarly, we did not observe a consistent pattern of changes in RMS intercept and slope at IC50, as well as RMS slope at IC80. Therefore, it seems that RMS does not provide reliable information about muscle recovery after muscle damage. These results supported the findings of other authors who either did not find any significant changes in RMS after EE (Sayers et al. 2001), or their RMS data was not statistically linear, and they did not perform further statistical analyses on RMS (Felici et al. 1997). However, our findings were not in line with the increased RMS observed by Berry et al. (1990) and Kroon and Naije (1991) after EE. The added variance introduced by electrode repositioning influences amplitude more than frequency parameters such as MDF (Merletti et al. 1995; Felici et al. 1997). This could result in observing different findings among different studies.

Study limitations

Because of its anatomical position, we monitored biceps brachii as the only elbow flexor in this study. There are some other elbow flexors that take part in elbow flexion. For example, as compared to biceps brachii, the brachialis muscle has a larger cross sectional area and is the prime elbow flexor. Therefore, it is possible that the functional

responsibilities of the elbow flexors have altered during isometric contractions performed in this study, and this might have a confounding effect on our findings.

Conclusion

We found a significant decrease in MDF during 50% and 80% of subject's MVT after a session of EE that did not fully recover to pre-exercise values up to 3 days after EE. This decrease could be related to a reduction in the recruitment of fast twitch fibres due to damage to these fibres. We also observed that compared to RMS, MDF was a more consistent parameter to reflect the changes in EMG during recovery from muscle damage.

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Chapter 5

Muscle Oxygenation after Downhill Walking-Induced Muscle Damage

As co-authors of the paper "Ahmadi S, Sinclair PJ, Davis GM. Muscle oxygenation after downhill walking-induced muscle damage. *Clin Physiol Funct I*, 2008, 28(1), 55-63.", we confirm that Sirous Ahmadi has made the following contributions:

- conception and design of the research
- data collection
- analysis and interpretation of the findings
- writing the paper and critical appraisal of content

PJ Sinclair…..……...……...……Date:……

GM Davis …….……..Date:……...

Abstract

The purpose of this study was to investigate changes in muscle oxygenation and blood flow within vastus lateralis after an exhaustive session of downhill walking (DW). Nine healthy males performed 40-min DW on a treadmill with a gradient of -25% and at walking velocity of 6.4 $km \bullet h^{-1}$. To increase the likelihood that DW would induce muscle damage, subjects were loaded with 5% of their body weight carried in a back pack. Before and after DW exercise on day 1 and over the next four days, maximum voluntary torque (MVT), subjects' perception of muscle soreness (SOR), plasma creatine kinase (CK) activity and myoglobin (Mb) concentrations, and muscle oxygenation (using near infrared spectroscopy; NIRS) within vastus lateralis were assessed. Repeated measures ANOVA revealed that MVT decreased while SOR and Mb concentration significantly ($p<0.05$) increased after DW, consistent with its effectiveness to evoke muscle damage. Resting tissue oxygen saturation increased immediately after DW, but recovered within 24 hours. During isometric contractions at 30%, 50% and 80% of MVC, oxygen desaturation and re-saturation kinetics became significantly faster than pre exercise values. The possible mechanism responsible for these changes might be increased resting muscle oxygen utilization after muscle damage due to an increased requirement for aerobic energy-demanding repair processes.

Key Words: Eccentric exercise, Muscle soreness, Oxygen uptake, Creatine Kinase, Near infrared spectroscopy, Oxygen saturation.

Introduction

It has been well documented that exhaustive downhill walking (DW) exercise, which involves repetitive eccentric contractions, may result in delayed onset muscle soreness and/or muscle damage (Farr et al. 2002; Marqueste et al. 2004). During eccentric exercise, muscle lengthening occurs despite the contraction induced by the central motor drive (Marqueste et al. 2004). Some of the known consequences of repetitive and unaccustomed eccentric exercise include long-lasting decreases in force generation, flexibility and range of motion. Increased muscular pain, tenderness, oedema and an efflux of intramuscular proteins into the blood stream are also secondary outcomes after eccentric exercise (Ebbeling and Clarkson 1989; Clarkson and Hubal 2002). However, the magnitude and/or the appearance of symptoms may vary depending on the training state of individuals, the duration, repetition and intensity of eccentric contractions, as well as the range over which muscle is extended.

Oxygen plays a crucial role in the life of human cells, and muscle oxygen consumption is increased during and immediately after exercise. Increased water content of the muscle, increased intramuscular pressures (Friden et al. 1983) and vasodilatation after eccentric exercise, may change the pattern of local blood flow and muscle oxygenation. In addition, possible changes in muscle fibre recruitment in eccentrically exercised muscles could also alter the pattern of tissue oxygenation. A selective damage of fast-twitch glycolytic fibres after eccentric exercise (Lieber and Friden 1988) may lead to the recruitment of fewer fast-twitch and greater slow-twitch fibres for a given task. Slow-twitch fibres possess greater oxidative capacity, and their recruitment for a given task may result in higher oxygen consumption compared to fast twitch fibres. Additionally, muscle damage resulting from eccentric exercise may increase the energy requirement and, therefore, resting muscle oxygen utilization for repair processes.

Previous researchers have attempted to explore the underlying mechanisms for the structural and biochemical changes resulting from eccentric exercise-induced muscle damage. The possible effects of eccentric exercise on muscle oxygenation and muscle blood flow (mBF) in humans, however, have not been thoroughly investigated, and in a

few studies that have investigated the changes in oxygen delivery and utilization within the exercised limb after eccentric exercise, incongruent results have been reported. For instance, Kano and co-workers (2004) observed significant alteration in capillary luminal shape and area for up to 3 days after eccentric exercise in rodents. In another animal study, Kano et al. (2005) also demonstrated that downhill running impaired muscle microcirculatory flows as well as the balance between oxygen delivery and consumption at the onset of such exercise. Furthermore, our previous work (unpublished observations) revealed significant increases in resting muscle oxygen saturation and decreases in oxygen desaturation and resaturation amount and rate for up to seven days following two concurrent sessions of biceps eccentric exercise. In contrast, Walsh and co-workers (2001) did not report any significant changes in oxygen utilization or local oxygen transport after a 30-min session of eccentric cycling in humans. Similarly, Laaksonen and colleagues (2006) did not find any significant changes in oxygen uptake after exhaustive stretch-shortening cycle exercise, although they observed a significant increase in mBF.

The disparity amongst previous studies, therefore suggested that further investigation into the effect of eccentric exercise during DW upon muscle oxygenation and associated measures was warranted. Accordingly, this study investigated the hypothesis that prolonged DW might alter muscle oxygenation and mBF. We employed near infrared spectroscopy (NIRS) to monitor the pattern of muscle oxygenation and blood flow before and for four days after a vigorous session of DW exercise. In addition to some parameters that have been employed in previous works (Walsh et al. 2001; Laaksonen et al. 2006), some new parameters of muscle oxygenation were assessed and monitored during isometric contractions at given intensities after downhill walking to further reveal changes in muscle oxygenation, if any.

Materials and Methods

Subjects: Nine male subjects (aged 27 ± 4.7 yr; body mass 70.7 ± 14.8 kg; stature 1.74 ± 0.67 m; mean ± SD) who had not participated in any form of muscular training for 6 months prior to commencing the study were recruited. The Human Research

Ethics Committee of the University of Sydney approved this study. All subjects were informed of the purpose, nature, and potential risks of their involvement, and gave written informed consent to participate. They were in healthy physical condition with no signs or symptoms of neuromuscular disorders, and they were requested to abstain from any exercise involving leg muscles for the duration of the investigations.

Experimental procedures: Prior to data collection, each subject reported to the laboratory to be acquainted with the experimental procedures and equipment set up. Testing sessions were performed over a period of 5 days and are described schematically in Figure 1. After subject placement, equipment set up and resting and exercising measurements (described subsequently) were completed, the subjects performed a session of DW exercise. Individuals were loaded with 5% of their body weight (3.5 ± 0.7) using a backpack filled with weights that were wrapped and stabilized to prevent any changes in subject coordination or balance during walking. Then, they performed a session of 40 min-downhill walking (phase 3; Figure 1) on a motorized treadmill at a gradient at -25% and an average velocity of 6.4 $km \bullet h^{-1}$. Three of the subjects required a short 5-min recovery during treadmill DW due to transient fatigue, and they completed the session thereafter.

Measurements

All measurements at rest and during isometric knee extension exercise were made in four discrete phases (Figure 1).

Resting measurements

Before (phase 1; Figure 1) and 30-min after (phase 4; Figure 1) the DW session on day 1 and for the next 4 days, certain measurements were made under resting conditions.

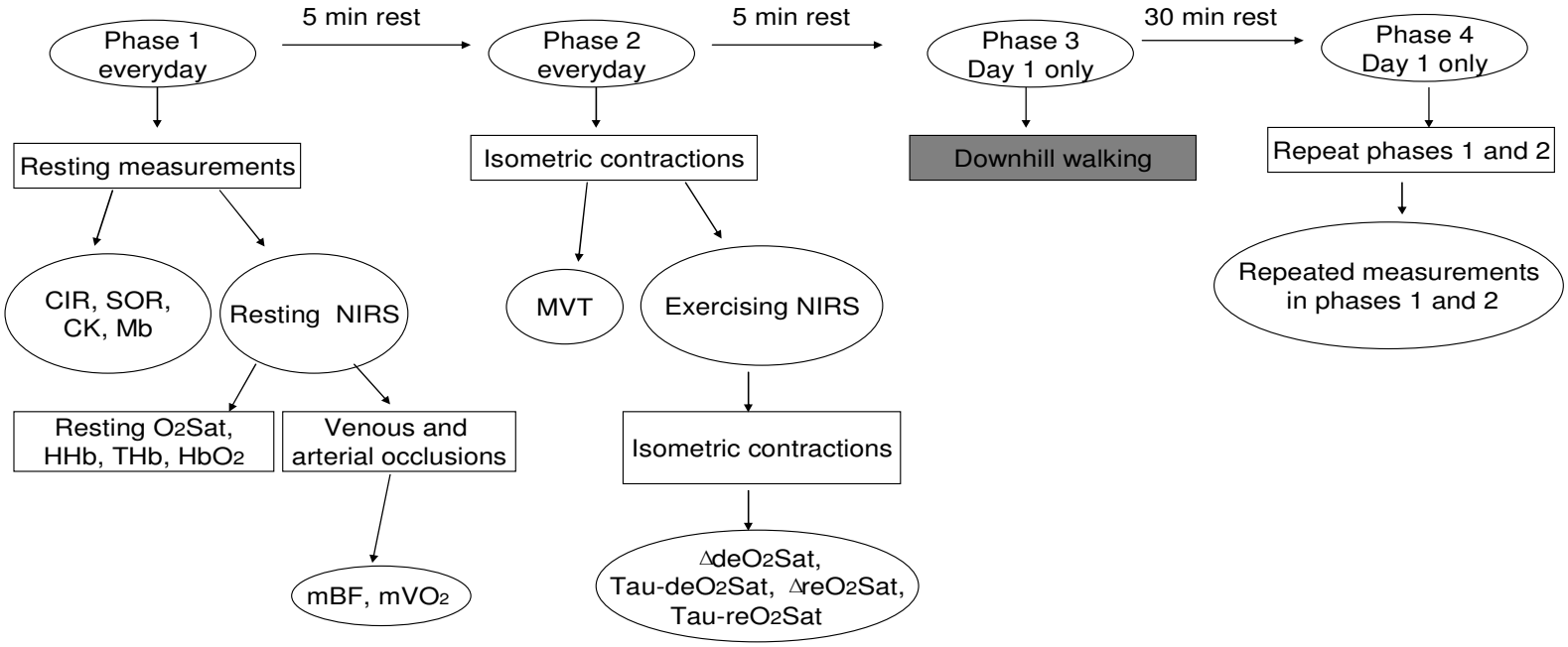

Figure 1: A schematic diagram of the experimental protocol employed in this study. Note: All abbreviations are described in the text, and some terms graphically described in Figure 2.

Creatine Kinase (CK) activity and Myoglobin (Mb) concentration: CK activity and Mb concentration were measured on day 1 before and after DW, and on days 3 and 5 at the beginning of each isometric testing session. At each sampling time, about 5 ml of venous blood was withdrawn from the antecubital vein, allowed to clot, and then centrifuged for 10-min. The serum was removed for analysis of CK activity activity within 24 hours, while the remainder was frozen for later analysis of Mb concentration. Plasma CK activity activity was assayed spectrophotometrically at 37°C using CK activity-NAC reagent kits (Thermo electron Corp., USA). Each plasma sample was assayed at least twice, until two assay values were within 10% of the lower value. The mean of the two values that were within 10% was used for statistical analyses. Serum Mb concentration was analysed using an enzyme immunoassay kit (MP Biomedicals, Orangeburg, NY, USA). Samples were analysed in duplicate. The mean coefficient of variation was 8.3% (n =36 samples).

Thigh circumference (CIR): CIR was measured at 6, 10 and 14 cm above the knee joint, with the leg relaxed in a neutral position. The average of three trials at each anthropometrical landmark was used for statistical analysis.

Perception of muscle soreness (SOR): A subjective rating of SOR was collected during each session. While standing, the subjects were asked to rate the integrated amount of pain experienced within their thigh during active flexion and extension of the knee joint on a scale from 1 to 10, where 1 corresponded to “no pain” and 10 was equated to “very, very painful”.

Skinfold thickness: Skinfold thickness was quantified to determine any relationship between these and resting NIRS-derived variables. The thickness of the skin at the middle portion of vastus lateralis (where the NIRS probe was to be placed), was measured using Harpenden skinfold calipers (John Bull; British Indicators, UK). These measurements were performed with subjects lying relaxed in a supine posture, three times on day 1 before commencing the experiment. The average of triplicate measurements was used for statistical analysis.

Near Infrared Spectroscopy: NIRS is a non-invasive, non-ionizing, real-time monitoring, continuous and direct method to determine oxygenation and hemodynamics in tissue. It enables the study of local differences in muscle O_2 consumption and delivery. The method has proved to be a valid monitor, useful for investigating the physiology of O_2 transport to tissue (for a review see Quaresima et al., 2003). NIRS is sensitive to changes in tissue oxygenation both at the level of the small blood vessels and capillaries and at the intracellular sites of O_2 uptake (Hampson and Piantadosi 1988). NIRS has also been shown to be a sensitive tool in the discrimination between normal and pathological states (Van Beekvelt et al. 2001).

NIRS measurements were obtained using a commercially available dual-channel frequency-domain system, (ISS OxiplexTS Oximeter, Model 96208, ISS Inc, Ill, USA). The NIRS system, which has been previously described (Quaresima et al. 2004), comprised an optical detector and a light source deploying wavelengths of 690 and 830 nm. Each sensor probe had four emitter positions (eight emitters) with emitter-detector distances ranging from 1.5 cm to 5.0 cm.

The NIRS system was calibrated using standard calibration blocks provided by the manufacturer (ISS Inc, Ill, USA). A rigid NIRS probe was attached to the middle part of subject's vastus lateralis in a longitudinal orientation. To prevent variations in placement of the NIRS emitter-detector, the angle and location of the probes were held constant during the test using a double-sided transparent adhesive tape. Similarly, the position of the NIRS probe was noted to the nearest millimetre and identified with a marker, to ensure identical placement on each subject for all the testing sessions across days. A light-impermeable cloth covered the probe to reduce room light interaction with near infrared signal. This frequency-domain NIRS system provided a direct estimation of muscle oxygen saturation (O_2Sat), oxyhaemoglobin (HbO_2), deoxyhaemoglobin (HHb) and total haemoglobin (THb)

Muscle O_2Sat, which is a commonly-derived parameter from NIRS studies, was the ratio of HbO_2 to THb (Boushel et al. 2001). O_2Sat reflects the dynamic balance between O_2 supply and O_2 consumption, and is independent of the path length of near-infrared

photons within the muscle tissue (Ferrari et al. 2004). Therefore, the major part of NIRS data presented in this paper was derived from the dynamic changes in O_2Sat, collected at rest, during super-diastolic and super-systolic occlusions, and during isometric contractions at 30%, 50% and 80% of subjects' maximum voluntary contraction (MVT). However, we also compared the resting values of THb, HbO_2 and HHb before versus after DW, and over the four days following exercise.

In addition to the traditional variables associated with NIRS, we derived some additional measures to express the absolute volume and rate of change (kinetics) of muscle oxygen saturation during arterial occlusion or exercise. These were previously described and employed in pilot studies (unpublished observations) and in a previous investigation of eccentric arm exercise (in review). Changes in muscle oxygen desaturation (ΔdeO_2Sat) were calculated as the difference in O_2Sat from resting levels to nadir (Figure 2) during exercise and arterial occlusion. The rate of change in ΔdeO_2Sat from rest to nadir provided a measure of the oxygen saturation kinetics, which we termed Tau-deO_2Sat (ΔdeO_2Sat divided by time from rest to the first point of nadir). Similarly, the muscle oxygen resaturation volume (ΔreO_2Sat) and kinetics (Tau-reO_2Sat) were calculated from the nadir of oxygen saturation during exercise or occlusion to the highest point during recovery (Figure 2).

The resting measurements for O_2Sat, THb, HHb and HbO_2 were continued for 3-min. Then, in order to estimate mBF at rest, two venous occlusions were applied above the belly of the quadriceps muscle (using a cuff air pressure inflated to 70mmHg), lasting 45 s with a 3 min recovery interval (Figure 2). Tissue mBF was then estimated by measuring the initial linear increase in THb (Kooijman et al. 1997; Boushel et al. 2000). Concentration changes of THb were expressed in micromole per second ($\mu M \bullet s^{-1}$), and converted into units of millilitre per minute per 100 gram of tissue ($ml \bullet min^{-1} \bullet 100g$), using an average Hb concentration of 140 $g \bullet l^{-1}$. The molecular weight of Hb (1 mole Hb is 64.458 kg) and the Hb to oxygen ratio (1:4) were also taken into account (Kooijman et al. 1997; Van Beekvelt et al. 2001). Tissue mBF at rest was calculated as the average during the two venous occlusions.

Then, after a further 3-min passive recovery, a super-systolic arterial occlusion (cuff air pressure inflated to 270 mmHg; Figure 2) was performed to elicit the minimum and maximum O_2Sat, the time-course of O_2Sat changes (kinetics), and to calculate muscle oxygen consumption (mVO_2). Arterial occlusion was continued until the O_2Sat reached a nadir lasting at least 30 s, usually after 5-8 min, then the cuff was released and subjects were prepared for the exercise trials. The initial linear decrease in HbO_2 was used to calculate mVO_2 (Kooijman et al. 1997; Van Beekvelt et al. 2002). The changes in HbO_2 given by the spectrophotometer are in micromoles, with a differential path length factor of 4.0 used to correct for scattering of photons in the tissue, and can be further converted to millilitres O_2 per minute per 100 g ($ml \bullet min^{-1} \bullet 100g$) tissue taking in account the certain held assumptions. The amount of oxygen that binds to haemoglobin (1 mole of HbO_2 binds 89.6 litres of oxygen, assuming STPD conditions) and the muscle density (1.04 kg per litre) was used to estimate mVO_2 (Kooijman et al. 1997; Van Beekvelt et al. 2002) .

A schematic diagram of the protocol employed in this study is shown in Figure 1 and a sample of NIRS O_2Sat measurements during rest, occlusions and isometric contractions is shown in Figure 2.

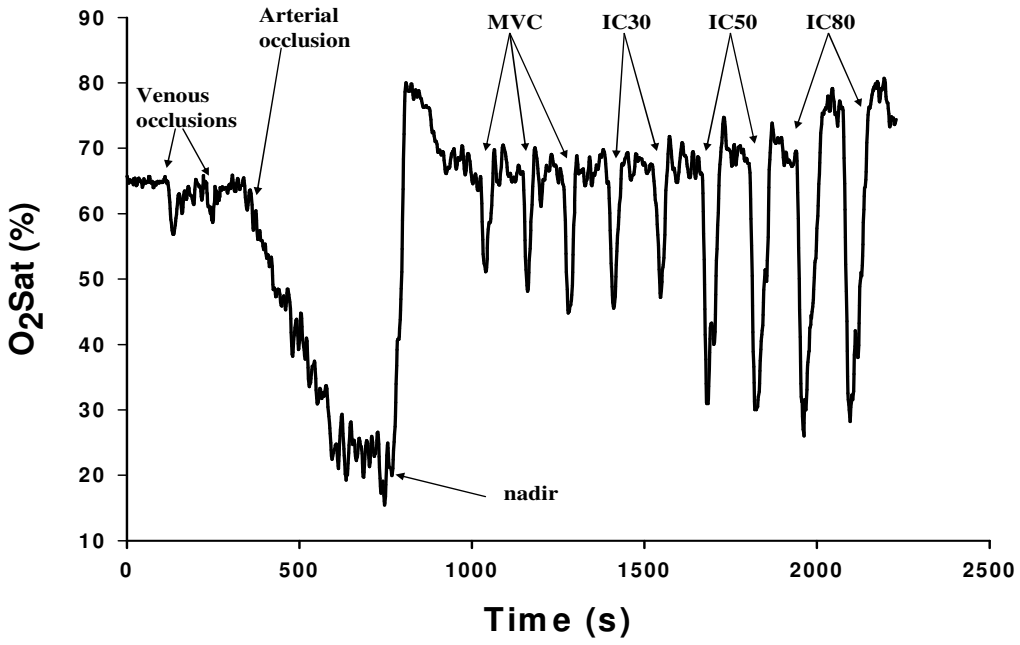

Figure 2: A sample of NIRS muscle oxygen saturation (O_2Sat) measurements obtained during each session. MVT refers to maximum voluntary contraction, and IC30, IC50, and IC80 refers to isometric contractions at 30%, 50%, and 80% of MVT, respectively.

Exercise assessments

Isometric knee extension MVT was assessed (phase 2; Figure 1) with the subject's hip and knee joints flexed at 90° using an isokinetic apparatus (Biodex System 2, Biodex, USA). Three 5 s repetitions were performed with 2-min of recovery between each maximal effort. The highest value was taken to represent the 100% MVT, and this was employed for statistical analyses.

After a further 5-min of recovery, isometric knee extensions were assessed (phase 2; Figure 1) at 30%, 50% and 80% of MVT (IC30, IC50 and IC80, respectively). The highest MVT from the first day before DW (baseline assessments) was used to calculate IC30, IC50 and IC80 for all subsequent sessions. During each session, subjects performed two isometric contractions at three intensities, each lasting 20 s with 3-min of passive recovery between efforts.

The previously-described isometric contractions were performed on both legs each day and within each session on every day. NIRS monitoring was performed continuously during isometric contractions, however the data from IC30, IC50 and IC80 were statistically analysed in this study. The order in which the legs were analysed was identical before and after DW, but the initial leg (left or right) was randomized between the subjects. The experimental sessions were set at the same time every day (in the mornings) for all subjects, and the temperature of the laboratory was maintained at 23-25°C.

Statistical Analyses

A t-test was performed on the two trials of isometric contractions of the same intensity (i.e. IC30, IC50 and IC80), and we observed that there were no significant differences between the two trials. Therefore, a two-way repeated-measures analysis of variance (RM ANOVA) was employed to assess whether there were significant changes in dependent variables over time (six assessments, with two assessments on day 1) and between legs (right and left), before and after downhill walking. When a significant leg or leg-by-time interaction effect was observed, univariate RM ANOVA was employed for each leg to probe for day-day effects and within-day effects (i.e. two assessments on day 1 before and after DW). For CK activity and Mb concentration, a one-way RM ANOVA was performed without leg main effect, since this measure represents a blood-borne enzyme. Statistical significance for a meaningful change was set at the 95% confidence level ($p < 0.05$). All values were reported as mean ± the standard error (SE) of the mean, because different variables represented different sampling times.

Results

There were no significant differences between two legs for any variables measured in this study. Accordingly, the data presented in this manuscript represents the mean value for both legs.

MVT declined significantly ($p<0.05$) after downhill walking on day 1 (1-POST) in both legs and remained lower than pre-downhill walking (1-PRE) on day 2 (Figure 3-a). In contrast, SOR increased significantly on days 2, 3, 4 and 5 (Figure 3-b). The univariate RM ANOVA displayed a non-significant trend for change in CK activity ($P= 0.084$), although it revealed a significantly higher CK activity on days 1-POST and 2 compared to 1-PRE (Figure 3-c). Similarly, Mb concentration increased significantly at 1-POST but it recovered to near resting values by day 3 (Figure 3-d). CIR (at all three anthropometric locations) revealed no changes within and between experimental days.

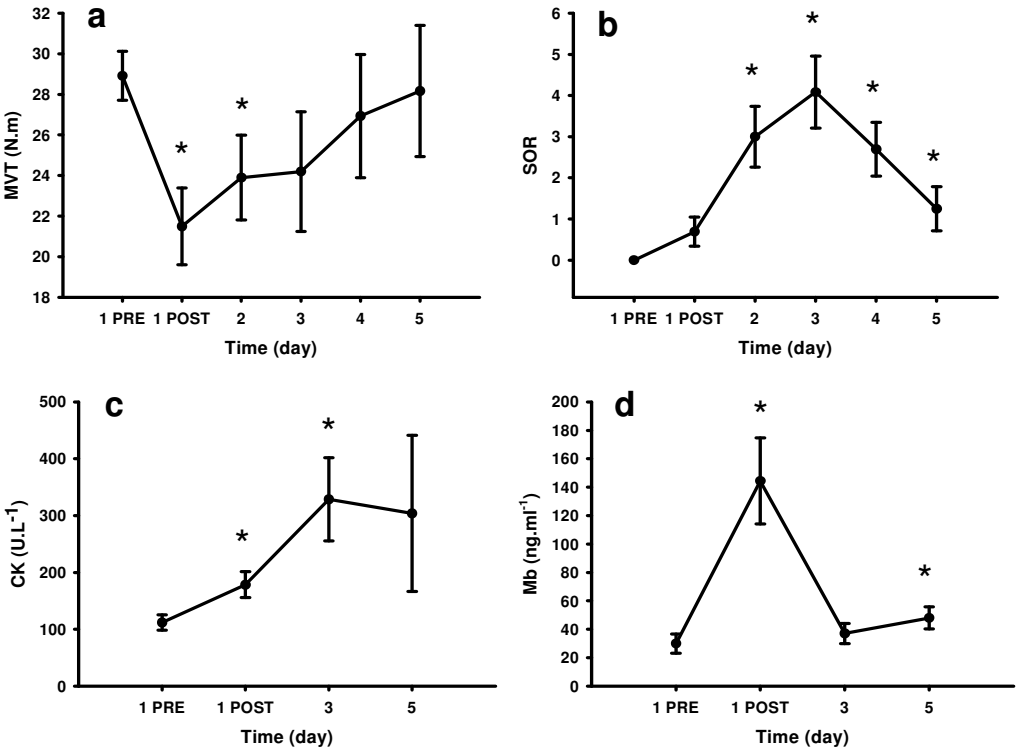

Figure 3: a) Maximum voluntary torque (MVT), b) Subjective perception of muscle soreness (SOR), c) Serum Creatine Kinase activity (CK), and d) Serum Myoglobin concentration (Mb) before and after downhill walking. * denotes significant ($p<0.05$) differences between day 1 before downhill walking and other days. Data are mean ± SE.

Although two-way RM ANOVA revealed a significant day main effect in resting O_2Sat and HbO_2, there were no significant differences between 1-PRE and any other sessions based on *a posteriori* univariate RM ANOVA analyses (Figure 4-a and 4-b, respectively). There was a significant day main effect for THb from the two-way RM

ANOVA, but *a posteriori* analysis yielded a difference between only day 3 and 1-PRE (Table 1). A slight trend for decrease of THb over the experimental days was noted (Table 1). For mBF, there was a significant day main effect increase, however the day-day differences were only significant between 1-PRE and 1-POST (Figure 4-c). Two-way RM ANOVA showed significant within subjects change in resting mVO_2, and the day-day comparisons revealed significant differences between 1-PRE and 1-POST and day 2 (Figure 4-d).

During super-systolic arterial occlusions, Tau-deO_2Sat increased significantly after downhill walking and remained higher than 1-PRE until day 5 (Table 1). On days 2 and 5, ΔreO_2Sat was significantly higher than 1-PRE (Table 1), but no significant change was revealed in ΔdeO_2Sat and Tau-reO_2Sat during super-systolic arterial occlusions (Table 1).

Table 1: NIRS derived variables at rest, during arterial occlusion (Art) and during isometric contractions at 50% and 80% MVT (IC50 and IC80, respectively). Since there were no significant differences between right and left legs, mean of both legs is presented.

		DAY 1 PRE	*DAY 1 POST*	*DAY 2*	*DAY 3*	*DAY 4*	DAY 5
IC50	**ΔdeO_2Sat #**	18.5 ± 1.7	24.5 ± 2.8 *	20.2 ± 2.3	20.3 ± 2.7	19.8 ± 1.9	19.3 ± 2.4
	Tau-deO_2Sat #	1.2 ± 0.09	1.9 ± 0.20 *	1.4 ± 0.16 *	1.3 ± 0.18	1.3 ± 0.12	1.3 ± 0.17
	ΔreO_2Sat	20.0 ± 1.9	22.6 ± 1.9	21.3 ± 2.1	19.6 ± 2.7	21.4 ± 2.4	21.2 ± 2.4
	Tau-reO_2Sat #	1.1 ± 0.15	0.9 ± 0.09 *	1.1 ± 0.13	1.0 ± 0.16	1.1 ± 0.17	1.1 ± 0.16
IC80	**ΔdeO_2Sat #**	24.6 ± 2.2	31.5 ± 2.1 *	27.3 ± 1.8 *	25.7 ± 2.7	27.0 ± 2.2	27.3 ± 2.5 *
	Tau-deO_2Sat #	1.8 ± 0.16	2.6 ± 0.15 *	1.9 ± 0.13 *	1.7 ± 0.18	1.9 ± 0.18	1.9 ± 0.20
	ΔreO_2Sat #	24.4 ± 2.1	27.2 ± 2.0 *	27.0 ± 1.8	25.4 ± 2.7	27.5 ± 2.2 *	29.2 ± 2.5 *
	Tau-reO_2Sat	0.83 ± 0.07	0.90 ± 0.09	0.95 ± 0.11	0.92 ± 0.11	0.94 ± 0.09	0.97 ± 0.10
Rest	**THb (µM) #**	82.3 ± 5.6	83.6 ± 6.3	81.4 ± 5.8	77.8 ± 4.5 *	77.3 ± 5.8	78.4 ± 5.4
Art	**ΔdeO_2Sat**	15.7 ± 1.8	16.3 ± 1.8	16.9 ± 1.7	16.4 ± 1.8	17.3 ± 1.7	16.6 ± 1.4
	Tau-deO_2Sat #	20.5 ± 2.4	18.6 ± 2.1 *	23.2 ± 2.5 *	20.5 ± 3.3 *	21.1 ± 2.2 *	24.8 ± 2.7
	ΔreO_2Sat #	19.5 ± 2.2	20.7 ± 1.8	21.5 ± 1.9 *	20.9 ± 1.7	22.5 ± 2.4	24.1 ± 2.2 *
	Tau-reO_2Sat	28.0 ± 2.2	29.8 ± 2.4	27.9 ± 2.2	26.4 ± 1.7	26.0 ± 1.9	25.5 ± 1.9

Note: ΔdeO_2Sat (percent) refers to changes in muscle oxygen desaturation, which were calculated as the difference in oxygen saturation from resting levels to nadir. Tau-deO_2Sat (percent•s^{-1}) refers to the rate of change in ΔdeO_2Sat from rest to nadir. ΔreO_2Sat (percent) refers to changes in muscle oxygen resaturation from nadir to peak recovery. Tau-reO_2Sat (percent•s^{-1}) refers to the rate of change in ΔreO_2Sat from nadir to peak recovery. # denotes significant within subject differences ($p<0.05$) observed on experimental days. * denotes significantly ($p<0.05$) different from day 1-PRE. Data are mean ± SE.

During isometric contractions, the following significant changes were observed. During IC30, ΔdeO_2Sat increased at 1-POST and until day 2, TaudeO_2Sat increased at 1-POST, and on days 2, 3, and 4, ΔreO_2Sat increased at 1- POST , and TaureO_2Sat increased on day 2 (Figure 5-a, b, c and d). Similar significant increases were observed during IC50 and IC80, which are shown in Table 1.

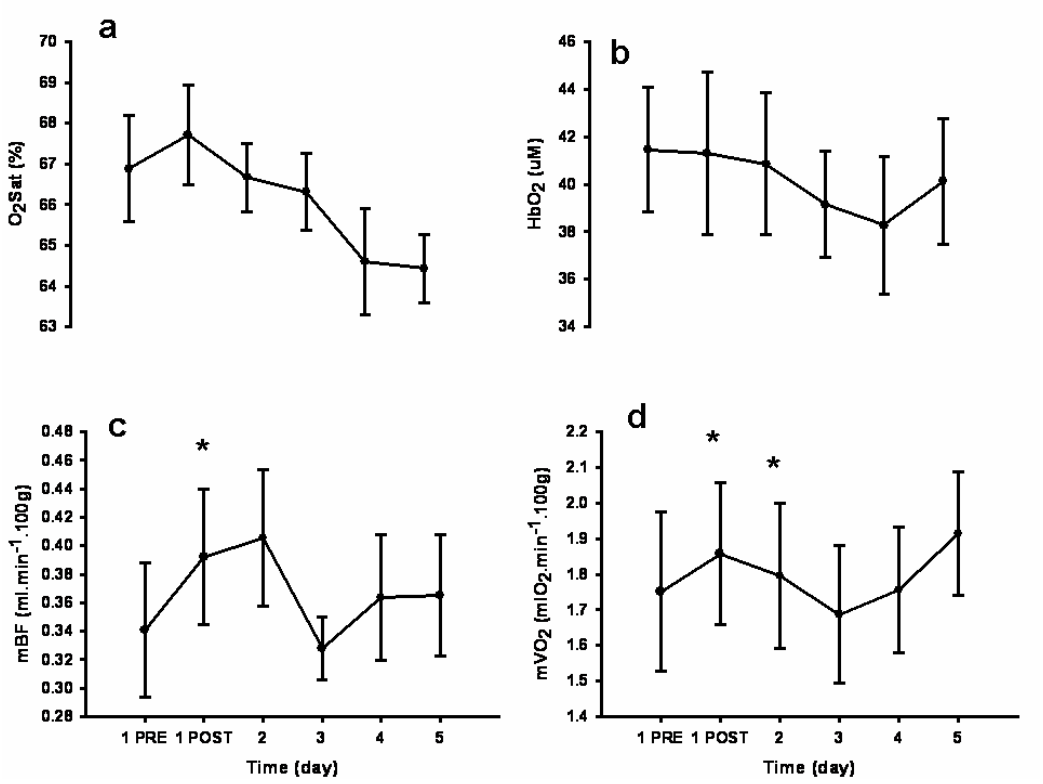

Figure 4: a) **Resting oxygen saturation (O_2Sat), b) oxyhaemoglobin (HbO_2), c) muscle blood flow (mBF), and d) muscle oxygen consumption (mVO_2) before and after downhill walking.** * denotes significant ($p<0.05$) differences between day 1 before downhill walking and other days. Data are mean ± SE.

Discussion

The downhill walking (DW) protocol deploying eccentric exercise in this study, was modified from a previous investigation (Farr et al. 2002), where it had been established to induce delayed onset of muscle soreness. Our findings were consistent with the results of that previous study. The following changes could testify to the evocation of exercise-induced muscle damage. However, it is worthy to note that our perception of muscle damage was based on indirect evidence such as MVT, Mb concentration CK activity, or physical signs, which are not categorical methods of determining muscle damage (e.g. muscle needle biopsy and MRI). Therefore, we have employed the term "muscle damage" with some caution.

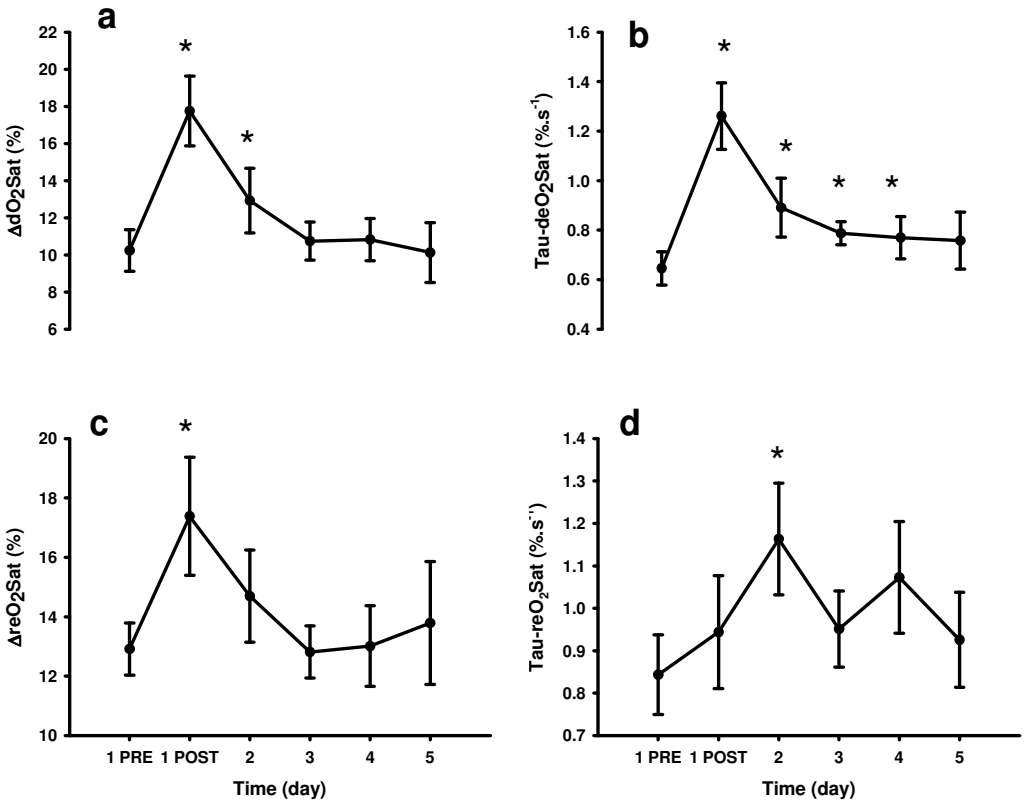

Figure 5: a) ΔdeO_2Sat, b) Tau-deO_2Sat, c) ΔreO_2Sat, and d) Tau-reO_2Sat during IC30 before and after downhill walking. Note: ΔdeO_2Sat (percent) refers to changes in muscle oxygen desaturation, which were calculated as the difference in oxygen saturation from resting levels to nadir. Tau-deO_2Sat (percent•s^{-1}) refers to the rate of change in ΔdeO_2Sat from rest to nadir. ΔreO_2Sat (percent) refers to changes in muscle oxygen resaturation from nadir to peak recovery. Tau-reO_2Sat (percent•s^{-1}) refers to the rate of change in ΔreO_2Sat from nadir to peak recovery. * denotes significant ($p<0.05$) differences between day 1 before downhill walking and other days. Data are mean ± SE.

Muscle force and other characteristics of muscle damage

MVT decreased significantly in both legs after DW and remained significantly lower than 1-PRE until day 3 (Figure 3-a). Up to a 25% decrease was observed in MVT after DW, although the degree and the onset of MVT loss varied amongst subjects. A prolonged decrement of muscle force output after exercise is one of the most valid and reliable indirect measures of muscle damage in humans (Warren et al. 1999). Although the day-day changes in CK activity were not significant, this variable was on 1-POST and day 2 significantly less than 1-PRE (Figure 3-c). The significant changes in MVT

and CK activity along with significant increments in Mb concentration and SOR demonstrated the effectiveness of our experimental protocol to evoke muscle damage.

Effects of downhill walking on NIRS-derived measures

Our findings revealed that there were significant day-day changes for O_2Sat, THb and HbO_2. As is shown in Figure 4-a and b (for O_2Sat and HbO_2, respectively) and Table 1 (for THb), these variables increased slightly at 1-POST and then decreased marginally over the following days. However, there were not any statistically significant differences between 1-PRE and other experimental days for these variables (except for the difference between 1-PRE and day 4 for THb). O_2Sat reflects the dynamic balance between O_2 supply and O_2 consumption (Ferrari et al. 2004). The immediate increments in O_2Sat, THb and HbO_2 after downhill walking could be simply due to the increased blood flow to the exercised limbs. This hyperaemic reaction could be observed after all types of exercise. However, the possible mechanism responsible for the modest decrements in resting O_2Sat, THb and HbO_2 over following days could be an increased resting muscle oxygen utilization due to probable muscle damage and a subsequent requirement of energy demanding repair processes (Walsh et al. 2001). This was supported by the significant increase in resting mVO_2 on 1-POST, and day 2 (Figure 4-d).

Although these results supported our initial hypothesis, they were not in line with the findings of our previous unpublished study (Ahmadi et al., in review), in which we observed a significant increase in resting O_2Sat for several days after arm eccentric exercise. The reason for this disparity was not clear. It could be partly attributed to the different experimental protocols applied between experiments. In our previous study, participants evoked 70 biceps brachii eccentric contractions, which elicited considerably greater muscle damage compared to the current investigation, as was evidenced by their larger changes in CK activity, MVT and SOR. Consequently, muscle oxygenation might have been affected differently. Furthermore, the muscle group that performed eccentric exercise in the previous investigation (arm muscles), was different from that of the current study (thigh muscles). In the previous study, the NIRS probe was placed over the belly of biceps brachii which was the main muscle affected by eccentric

exercise. In the current study, however, NIRS probe was secured on vastus lateralis, which was one of the few muscles involved in downhill walking that could be damaged. Previous studies have reported that there are different levels of muscle oxygenation in different agonist muscles performing similar exercises (Azuma et al. 2000; Hiroyuki et al. 2002). In addition, a particular tissue region monitored by NIRS might differ in blood flow and metabolic rate from the average value of a particular body segment (Boushel et al. 2001).

Our findings revealed that mBF increased after downhill walking, and slowly recovered over the following days (Figure 4-d.). During exercise, the vascular portion of active muscles is considerably increased by the dilation of local arterioles (McArdle et al. 1986). Therefore, an increase in mBF during and immediately after any type of exercise would be an expected finding. However, a prolonged increase in mBF after eccentric exercise might be due to inflammatory responses provoked by muscle damage and/or soreness (Laaksonen et al. 2006). Our finding was supported by the results of Sbriccoli et al. (2001) and Laksonen et al. (2006) who detected a marked increase in local blood flow for 2-4 days after eccentric exercise.

In this study, a prolonged increase was observed in Tau-deO_2Sat and ΔreO_2Sat during super-systolic arterial occlusions after downhill walking (Table 1). Similarly, during isometric contractions of different exercise intensities (i.e. IC30, IC50 and IC80), all NIRS-derived O_2 kinetics including ΔdeO_2Sat, ΔreO_2Sat, Tau-deO_2Sat and Tau-reO_2Sat (except for ΔreO_2Sat during IC50 and Tau-reO_2Sat during IC80), increased significantly after DW exercise (Figure 5 and Table 1). These findings strongly suggest that during isometric contractions, after a vigorous, prolonged downhill walking session, muscles were desaturated and resaturated to greater degrees and with higher rates compared to pre-downhill walking resting conditions. Consequently, based on the assumption that O_2sat represented the balance between oxygen delivery and oxygen uptake (Ferrari et al. 2004), these findings may further indicate that mVO_2 has increased due to exercise-induced muscle damage. We have tentatively concluded that after a single session of downhill walking, the vastus lateralis muscle used more oxygen to perform a given task

(isometric contraction at given intensities) within a certain time (20 s) compared to undertaking the same task from a basis of resting conditions.

Factors such as O_2 delivery status (e.g., blood flow), presence of nitric oxide, and kinetics of high-energy phosphate hydrolysis' products (e.g. PCr depletion kinetics and creatine kinase activity) could affect kinetics of the initial mVO_2 increase at the onset of exercise (Jones and Poole 2005). The possible increments in mBF might theoretically reduce the chance of an additional fall in oxygen saturation during exercise. However, the increased intramuscular pressure and, therefore, the augmented mechanical pressure on the exercising muscle resulted from isometric contractions might decrease blood supply and consequently oxygen saturation for exercising fibres. Kano and colleagues (2005) demonstrated that downhill running in rats impaired both microcirculatory flow and the balance between oxygen delivery and oxygen utilization at the onset of exercise, as evidenced by an accelerated fall of microvascular oxygen pressure. They proposed that the lowered oxygen pressure head during the first 20-40 s of muscle contractions may result in an impaired blood myocyte oxygen diffusion. The accelerated fall in microvascular oxygen pressure during the initial stage of contractions in Kano and associates' investigation is similar to the faster oxygen desaturation that we observed during isometric contractions.

Muscle fibre recruitment may also affect oxygen consumption. Selective damage of fast-twitch glycolytic fibres after eccentric exercise (Lieber and Friden 1988) could decrease and increase the recruitment of fast-twitch and slow-twitch fibres for a given task, respectively. Therefore, since slow-twitch fibres possess greater oxidative capacity, their extended recruitment for a given task could increase mVO_2.

However, our findings were not in accordance with the results of Walsh and co-workers (2001) and Laaksonen et al. (2006), who did not find any significant changes in muscle oxidative function after eccentric exercise. The disagreement between our results and those of Walsh et al. (2001) could be due to differences between the methods of data acquisition in the two studies. Walsh et al. (2001) collected their NIRS-derived data during rest and circulatory occlusions and then measured VO_2 (using Douglas bags)

during a 10-min concentric cycling. In the current study, however, in addition to the resting measurements, we collected NIRS-derived data during short-term (20 s) isometric contractions. It is assumed that those factors which regulate or affect the kinetics of VO_2 during primary or fast component (initial increase) could be different from those which affect the slow component (for review see Jones and Poole, 2005 and Grassi, 2000). Therefore, muscle oxygenation kinetics during 10- min cycling could be different from that of during 20s isometric contractions. The training status of participants could also be another factor to cause the disparity of findings. Walsh et al (2001) recruited Physical Education students for their study, while our subjects were not physically active in any regular exercise program for 6 months before commencing the study. Hence, a larger extent of muscle damage and, therefore, different levels of adaptations in oxygenation kinetics could be expected in our study. Additionally, Walsh and colleagues (2001) used eccentric cycling to induce muscle damage, which is an unusual type of exercise where eccentric activity occurs without concentric activity and where the impact force is relatively low. In contrast, during downhill waking both eccentric and concentric activities occur and the impact force is much higher (Walsh et al. 2001).

Similarly, Laaksonen and colleagues (2006) did not observe a significant change in muscle oxygen consumption, although they detected an increased muscle blood flow three days following a session of stretch shortening cycle. This was in contrast to the well-established finding of Johnson and Saltin (1985), who demonstrated a close positive correlation between mBF and mVO_2. Findings of Laaksonen et al. (2006) were not also in line with those of our study. The methodological differences in data acquisition and analyses could have contributed to this divergence. For example, Laaksonen and associates (2006) measured oxygen consumption using positron emission tomography during low intensity (~8% of MVT) dynamic contractions. Therefore, their results do not reflect the mBF and oxygen consumption responses during higher exercise intensities (Laaksonen et al. 2006), such as those performed in the current study.

Conclusion

Collectively, the findings of our study supported the hypothesis that downhill walking-induced muscle damage may affect or be affected by muscle oxygenation kinetics, as evidenced by the changes in resting and exercising muscle oxygenation. The current study revealed significant and prolonged increments in resting muscle oxygen consumption, as well as in oxygen desaturation and resaturation kinetics during isometric contractions of different intensities. The possible mechanisms responsible for the above changes could be impairments in microcirculatory flow, possible increases in slow-twitch fibre recruitment, as well as increments in resting muscle oxygen utilization due to probable muscle damage and a subsequent requirement of energy demanding repair processes. Our findings were in line with some previous animal studies (Kano et al. 2004, 2005), but dissimilar, at least for some part, to some human studies (Walsh et al. 2001 and Laaksonen et al. 2006). Therefore, investigation in humans is warranted to further assess the modifications in muscle oxygenation kinetics evoked by exercise-induced muscle damage.

Acknowledgment

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Chapter 6

Muscle Oxygenation Following Concentric Exercise

As co-authors of the paper "Ahmadi S, Sinclair PJ, Davis GM. Muscle Oxygenation Following Concentric Exercise. *Isokin Exer Sci*: 2007, 15(4), 309-319.", we confirm that Sirous Ahmadi has made the following contributions:

- conception and design of the research
- data collection
- analysis and interpretation of the findings
- writing the paper and critical appraisal of content

PJ Sinclair…..……...……Date:……

GM Davis …….……..Date:……...

1-Abstract

We investigated the hypothesis that a session of maximal concentric contraction exercise (CE) might impair muscle oxygenation and muscle blood flow. Ten healthy males performed a single bout of 70 maximal elbow flexion CE. Before and after CE on day 1 and over the next two days, maximum voluntary isometric torque (MVT), plasma creatine kinase activity (CK) , serum myoglobin, elbow joint range of motion (ROM), subjects' perception of muscle soreness (SOR), and muscle oxygenation kinetics (using near infrared spectroscopy; NIRS) within biceps brachii were assessed. MVT and ROM decreased while SOR increased significantly after CE ($p<0.05$), but recovered within the next day. There were not any significant changes in CK or myoglobin. Resting muscle oxygen saturation, oxyhaemoglobin and muscle oxygen uptake increased, and deoxyhaemoglobin decreased significantly after acute CE, and recovered by day 2. Although the changes in NIRS-derived variables were not significant in most conditions during isometric contractions of different intensities (i.e. 30%, 50% and 80% of MVT), there was a consistent pattern of change for those variables (i.e. decreasing immediately after CE, recovering on day 2 and increasing on day 3). Interestingly, the pattern of change in NIRS parameters mirrored CE-induced changes of MVT. *In conclusion, c*oncentric exercise did not produce prolonged changes in muscle oxygenation. The changes observed in NIRS-derived variables under resting condition may reflect excess post-exercise oxygen consumption.

Key Words: Oxygen saturation, Blood flow, Creatine Kinase, Myoglobin, Near infrared spectroscopy, Maximum voluntary contraction

2- Introduction

The term ‘concentric contraction’, which has been suggested should be replaced by the phrase ‘shortening action’ [1], is an inherent component of many daily activities, including athletic movements, whereby the muscle shortens while contracting [1, 2]. As opposed to eccentric (lengthening) movements, concentric exercise (CE) usually does not evoke exercise-induced muscle damage or delayed onset of muscle soreness. In a non-fatigue condition, concentric force was less than eccentric, and the level of force decrements after fatiguing exercise was less for concentric compared to eccentric contractions [3]. However, similar patterns of changes in motor unit activity and metabolism have been observed in human skeletal muscle during and after eccentric and concentric contractions [4].

Adequate supply of oxygen is necessary for the normal function of all cells [5]. Oxygen delivery and utilization to the muscle during different types of exercise has been extensively investigated in both animals and humans [6-9]. Furthermore, oxygenation kinetics during recovery immediately after exercise has been investigated (see Gaesser and Brooks, 1984), and it is known that muscle oxygen consumption (mVO_2) could remain high for up to few hours after exercise depending on the exercise type, duration and intensity, as well as the individuals conditions [10]. Also, during recovery from exercise the estimated muscle capillary blood flow kinetics were biphasic, and the overall kinetics of capillary blood flow were slower than the estimated muscle oxygen uptake [11]. However, the possible long-term effects of concentric exercise on local muscle oxygenation have not been comprehensively studied, and the available data from humans have been mostly obtained by measuring pulmonary gas exchanges rather than monitoring muscle oxygenation at the site of exercised muscle.

Traditionally, it was believed that lactic acid was the main determinant of excess post-exercise oxygen consumption (EPOC) [17]. However, it is currently believed that other factors such as catecholamines, calcium^{2+} and elevated tissue temperature might affect the oxygen consumption following exercise (see Gaesser and Brooks, 1984). Increased intramuscular pressures, as well as increased water content of muscle, resulting from

exhaustive exercise [18], might also affect the pattern of muscle oxygenation during and after exercise. Our previous studies (Ahmadi et al, in review) have observed that eccentric exercise alters the pattern of muscle oxygenation for several days afterwards. However, it might be speculated that heavy concentric contractions would produce similar results. Therefore, in this study, we investigated the effect of maximal repetitive concentric contractions on muscle oxygenation and blood flow.

We used near infrared spectroscopy (NIRS) to monitor the oxygenation of biceps brachii muscle before and for two days after a session of maximal concentric exercise. NIRS is a simple and non-invasive tool for monitoring tissue oxygenation in humans during both static and dynamic muscle contractions [12]. NIRS theory, its applications and limitations have been described previously [13, 14] and NIRS-derived variables have been employed to estimate muscle blood flow (mBF) and mVO_2 [15, 16].

3- Material and Methods

Subjects: Ten healthy male subjects with mean (± SD) age 25.7 ± 4.5 yr, body mass 73.8 ± 8.6 kg and height 1.78 ± 0.07 m, who had not participated in any kind of systematic muscular training for 12 months before commencing the study took part in the experiment. The Human Research Ethics Committee of the University of Sydney approved the study. All subjects were informed of the purpose, nature, and potential risks of their involvement, and gave written informed consent to participate. The subjects were in healthy physical condition with no signs or symptoms of neuromuscular disorders, and they were requested to abstain from any exercise involving their arm muscles for the duration of the study.

Study design: Four testing sessions were performed over a period of 3 days (two sessions on day 1 and one session on days 2 and 3; Figure 1). Each individual was requested to attend the laboratory four times, and on their first visit they were habituated to the technical procedures used. Each subject's biceps brachii of the non-dominant arm performed CE (Exercise), whereas the biceps brachii of the dominant arm was employed as non-exercise limb (Control). The following assessments were performed

everyday on both Control and Exercise limbs: Plasma Creatine Kinase (CK) activity and myoglobin (Mb) concentration, elbow range of motion (ROM), upper arm circumference (CIR), subjects' perception of muscle soreness (SOR), maximum voluntary isometric torque (MVT) and NIRS-derived muscle oxygenation measures at rest and during isometric contractions at different intensities. However, on day one after the above assessments (pre-CE assessments) on both Control and Exercise limbs, subjects performed concentric contractions (described below) with their Exercise arm. Then after 30-min passive rest, the pre-CE assessments were repeated (post-CE assessments) on Exercise and Control arms, respectively. The experimental sessions were set at the same period every day (in the mornings) for each subject, and room temperature was set between 23°C to 25°C for all subjects. The experimental protocol measured one arm at a time and subjects were unaware which limb was going to be assessed first. A schematic diagram of the experimental protocol for day 1 is presented in Figure 1. On days 2 and 3 the same set of assessments performed in pre-CE session (on day 1) were employed.

Concentric Exercise protocol: On the first day of testing sessions, subjects performed two sets of 35 maximal voluntary CE (each repetition comprised 5 s of concentric contraction and 12 s of passive recovery) with the elbow of their non-dominant arm placed in an isokinetic apparatus (Biodex System 2, Biodex, USA). Participants were requested and encouraged both verbally and visually, to maximally pull up the Biodex's handle from an elbow-extended (0°) to an elbow-flexed (130°) position at a preset angular velocity of 30 $deg \cdot s^{-1}$. The apparatus brought subject's arm back to the elbow-extended position after each concentric contraction. The two CE sets were separated by a 5-min recovery interval.

Measurements: Before and 30 min after the concentric contractions on day 1 and for the next two days, the following measurements were made on Exercise and Control arms.

Serum Creatine Kinase (CK) and Myoglobin (Mb): CK activity and Mb concentration were measured at the beginning of each session. At each sampling time, 5

ml of venous blood was withdrawn from the antecubital vein, allowed to clot, and then centrifuged for 10-min. The serum was removed for analysis of CK activity within 24 hr, while the remainder was frozen for later analysis of Mb concentration. Plasma CK activity was assayed spectrophotometrically at 37°C using CK -NAC reagent kits (Thermo electron CORP., USA). Each plasma sample (n = 40 samples) was assayed at least twice, until two assay values were within 10% of the lower value. The mean of the two values that were within 10% was used for statistical analyses. Serum Mb concentration was analysed using an enzyme immunoassay kit (MP Biomedicals, Orangeburg, NY, USA). Samples were analysed in duplicate. The mean coefficient of variation was 8.06% amongst samples (n = 40 samples).

Elbow range of motion (ROM): Subjects were instructed to stand beside a whiteboard in a relaxed position with their arm relaxed (extended position). At this time, an experienced investigator marked the locations of shoulder (Acromion), elbow (Olecranon) and wrist (Styloid process) on the whiteboard and measured the angle using a goniometer. The subject then flexed his forearm while the elbow and shoulder joints were kept constant with the assistance of another investigator. The new position of wrist was marked again on the whiteboard (flexed position) and the angle was measured. The difference between extended and flexed positions was taken into account as ROM. This was repeated 3-times and the average of the 3 ROM was used for statistical analyses.

Arm circumference (CIR): CIR was measured at 4, 6, 8, and 10 cm above the elbow joint, while allowing the arm to hang down by the side. The average of the three trials for ROM and CIR was used for statistical analyses.

Perception of muscle soreness (SOR): A subjective rating of SOR was performed during each session using a 7-point categorical scale, where 1 corresponded to “no pain” and 7 to “very, very painful”. While standing, the subjects were instructed to gently palpate their upper arm during full range of motion biceps curls and then choose the number that corresponded to their perceived level of soreness [19].

Muscle oxygenation and blood flow: Muscle oxygenation and blood flow were estimated using a frequency-domain multidistance near-infrared spectroscopy system (OxiplexTS, ISS, Champaign, IL, USA). The NIRS system comprised an optical detector and a light source deploying wavelengths of 690 and 830 nm. Each sensor probe had four emitter positions (eight emitters) with emitter-detector distances ranging from 1.5 cm to 5.0 cm.

The NIRS system was calibrated prior to each testing session, according to the manufacturer's (ISS Inc, Ill, USA) standard procedures. A NIRS probe was attached to the middle part of subject's biceps brachii muscle longitudinally. To prevent variations in placement of the NIRS emitter-detector, the angle and location of the probes were held constant during the test using a double-sided adhesive tape. Similarly, the position of the NIRS probe was noted to the nearest millimetre and identified with a marker, to ensure identical placement on each subject for all the testing sessions. A light-impermeable cloth covered the probe to reduce room light interaction with near infrared signal. This frequency-domain NIRS system provided a direct estimation of tissue oxyhaemoglobin saturation (O_2Sat), oxyhemoglobin (HbO_2), deoxyhaemoglobin (HHb) and total haemoglobin (THb). O_2Sat, which is a commonly-derived parameter from NIRS studies, was the ratio of HbO_2 to THb [14].

The major part of NIRS data presented within this manuscript was obtained from the changes in O_2Sat, collected at rest, during super-diastolic and super-systolic occlusions, and during isometric contractions at 30%, 50% and 80% of maximum torque. However, we also compared the resting values of THb, HbO_2 and HHb before and after CE, and over three days following exercise.

After 3-min of NIRS data collection during rest, to estimate resting mBF, two venous occlusions were applied above the belly of the biceps muscle (using a cuff air pressure inflated to 70 mmHg), each lasting 45 s with a 2-min recovery interval. mBF then was estimated by measuring the initial linear increase in THb [16, 20]. Concentration changes of THb were expressed in micromolar per second, and converted into units of millilitre per minute per 100 millilitres of tissue, using an average Hb concentration of

140 g•l^{-1} for male subjects. The molecular weight of Hb (1 mole Hb weighs 64.458 kg) and the Hb to oxygen ratio (1:4) were also taken into account [16, 21]. mBF during rest was calculated as the average during the two venous occlusions.

Then, after a further 3-min of recovery, a super-systolic arterial occlusion (cuff air pressure inflated to 270 mmHg) was performed to record the minimum and maximum O_2Sat, the time-course of O_2Sat changes (kinetics), and to calculate local mVO_2. Arterial occlusion was continued until the O_2Sat decreased and reached a nadir lasting at least 30 s, usually after 5-8 min, then the cuff was released and subjects were prepared for the exercise trials. The initial linear increase in HHb was used to calculate mVO_2 [15, 16]. The changes in HHb given by the spectrophotometer are in micromolar per second, with a differential path length factor of 4.0 used to correct for scattering of photons in the tissue, and can be further converted to millilitres O_2 per minute per 100 g tissue, taking in account the following assumptions. The amount of oxygen that binds to haemoglobin (1 mole of HbO_2 binds 89.6 litres of oxygen, assuming STPD conditions) and the muscle density (1.04 kg•l^{-1}) was used to estimate mVO_2 [15, 16] .

In addition to the traditional variables associated with NIRS, we derived some additional measures to express the absolute volume and rate of change (kinetics) of muscle oxygen saturation during arterial occlusion or exercise. Changes in muscle oxygen desaturation (ΔdeO_2Sat) were calculated as the difference in O_2Sat from resting levels to nadir during exercise and arterial occlusion. The rate of change in ΔdeO_2Sat from rest to nadir provided a measure of the oxygen saturation kinetics, which we termed Tau-deO_2Sat (ΔdeO_2Sat divided by time from rest to nadir). Similarly, the muscle oxygen resaturation volume (ΔreO_2Sat) and kinetics (Tau-reO_2Sat) were calculated from the nadir of oxygen saturation during exercise or occlusion to the highest point during recovery.

Isometric contractions: Maximum voluntary isometric contraction torque (MVT) was assessed on Exercise and Control limbs with the subject's elbow joint set to 90° and shoulder at 45° using the Biodex system. Three 5 s repetitions were performed with 2-

min of recovery between each maximal effort. The highest value was taken to represent the 100% MVT, and was employed for statistical analyses.

After a further 5-min recovery, isometric biceps contractions were performed at 30%, 50% and 80% of MVT (IC30, IC50 and IC80, respectively). The highest MVT from the first day before CE (baseline assessments) was used to calculate IC30, IC50 and IC80 for all subsequent sessions. On each session, subjects performed two isometric contractions at each of the three intensities, each lasting 20 s with 3 min of passive recovery between efforts. Participants could observe their efforts (force output on a computer monitor) to reach and maintain the average exercise intensity requested by the investigator (30%, 50%, 80%, or 100% of MVT). The IC30, IC50 and IC80 assessments were performed every day in order to assess muscle oxygenation (using NIRS) during exercise at given intensities.

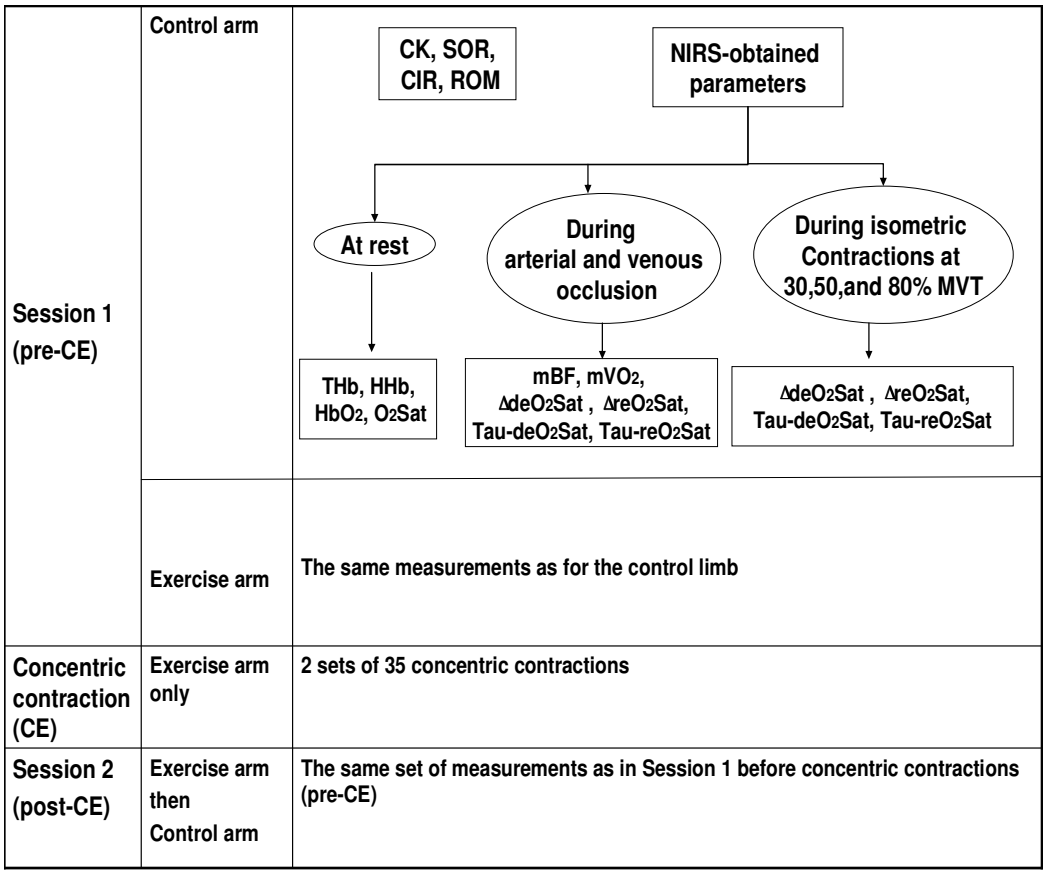

Figure 6: A schematic diagram of the experimental protocol employed on day 1 in this study. Note: All abbreviations are described in the text.

4- Statistical Analyses

A t-test revealed that there was no significant different between the two trials for each intensity (IC30, IC50 or IC80). Therefore mean of the two trials were applied for the following statistical analyses. A two-way repeated-measures analysis of variance (RM ANOVA) was employed to assess whether there were significant changes in dependent variables over time (four assessments, with two assessments on day 1) and between arms (Control, Exercise), before and after CE. In these RM ANOVA analyses, the within-subjects factor was time (4 assessments), and the between-subjects factor was arm (Control versus Exercise). When a significant arm, time or arm-by-time effect was observed, univariate (one-way) RM ANOVA (main effect factor being time) was employed for each arm to test for day-day effects and within-day effects (i.e. two

assessments on day 1 before and after CE). For CK activity and Mb concentration, a one-way RM ANOVA was performed without an arm main effect, since these measures represented circulating enzymes. Statistical significance for a meaningful change was set at the 95% confidence level ($p< 0.05$). All values were reported as mean ± standard error (SE).

5- Results

There was an arm-by-time interaction effect for MVT. Univariate ANOVA for each arm revealed a trend for a decrease in MVT in the post-CE session on day 1 within the Exercise limb ($P = 0.056$), which had recovered on day 2. MVT increased significantly on day 3 in both arms (Figure 2-a). There was not any arm-by-time interaction effect for CIR (at all anthropometrical locations), SOR (Figure 2-c) or ROM (Figure 2-b). However, there was a significant time effect (representing day-day changes) for CIR, which showed a trend for an increase in both arms (Table 1). Additionally, CK activity (Figure 2-d) and Mb concentration (Table 1) did not change significantly after CE.

Table 1: Summary of the changes in some of the variables measured during rest and exercise.

Variable	DAY 1 PRE	DAY 1 POST	DAY 2	DAY 3
IC30 ΔdeO_2Sat (percent)	16.1 ± 2.9	13.9 ± 1.6	13.6 ± 2.7	15.9 ± 3.1
IC30 Tau-deO_2Sat (percent•s^{-1})	1.1 ± 0.2	1.1 ± 0.11	1.0 ± 0.18	1.2 ± 0.22
IC30 ΔreO_2Sat (percent)	14.5 ± 2.7	11.6 ± 1.6	12.5 ± 2.8	14.4 ± 2.6
IC30 Tau-reO_2Sat (percent•s^{-1})	0.98 ± 0.13	0.79 ± 0.13	0.99 ± 0.21	1.1 ± 0.15
IC80 ΔdeO_2Sat (percent)	27.8 ± 4.1	19.9 ± 1.8	28.0 ± 4.6	31.3 ± 4.6
IC80 Tau-deO_2Sat (percent•s^{-1})	2.49 ± 0.38	1.93 ± 0.14	2.54 ± 0.41	2.57 ± 0.40
IC80 ΔreO_2Sat (percent) #	25.2 ± 2.1	14.2 ± 2.1*	23.8 ± 2.9	28.4 ± 2.2
IC80 Tau-reO_2Sat (percent•s^{-1})	0.98 ± 0.14	0.87 ± 0.15	1.03 ± 0.15	1.20 ± 0.18
THb (µM)	67.9 ± 5.3	69.1 ± 5.8 *	64.0 ± 5.5	65.6 ± 5.1
HHb (µM) #	18.4 ± 1.6	9.6 ± 0.9	16.7 ± 1.5	17.6 ± 1.6
Mb concentration (ng.ml^{-1})	35.2 ± 4.5	43.0 ± 7.3	39.0 ± 8.3	41.1 ± 6.4
CIR (cm)	26.2 ± 0.69	26.7 ± 0.69 *	26.6 ± 0.72 *	26.6 ± 0.71 *

Note: ΔdeO_2Sat (percent) refers to changes in muscle oxygen desaturation, which were calculated as the difference in oxygen saturation from resting levels to nadir. Tau-deO_2Sat (percent•s^{-1}) refers to the rate of change in ΔdeO_2Sat from rest to nadir. ΔreO_2Sat (percent) refers to changes in muscle oxygen resaturation from nadir to peak recovery. Tau-reO_2Sat (percent•s^{-1}) refers to the rate of change in ΔreO_2Sat from nadir to peak recovery. IC30 and IC80 refer to isometric contraction at 30% and 80% of MVT, respectively. THb, HHb, Mb concentration and CIR refer to total hemoglobin, deoxy haemoglobin, serum myoglobin and arm circumference, respectively. # denotes significant arm-by-time differences ($p<0.05$) observed on experimental days. * denotes significant ($p<0.05$) differences between day 1 pre-CE and other days. Data are mean ± SE, for Exercise arm only.

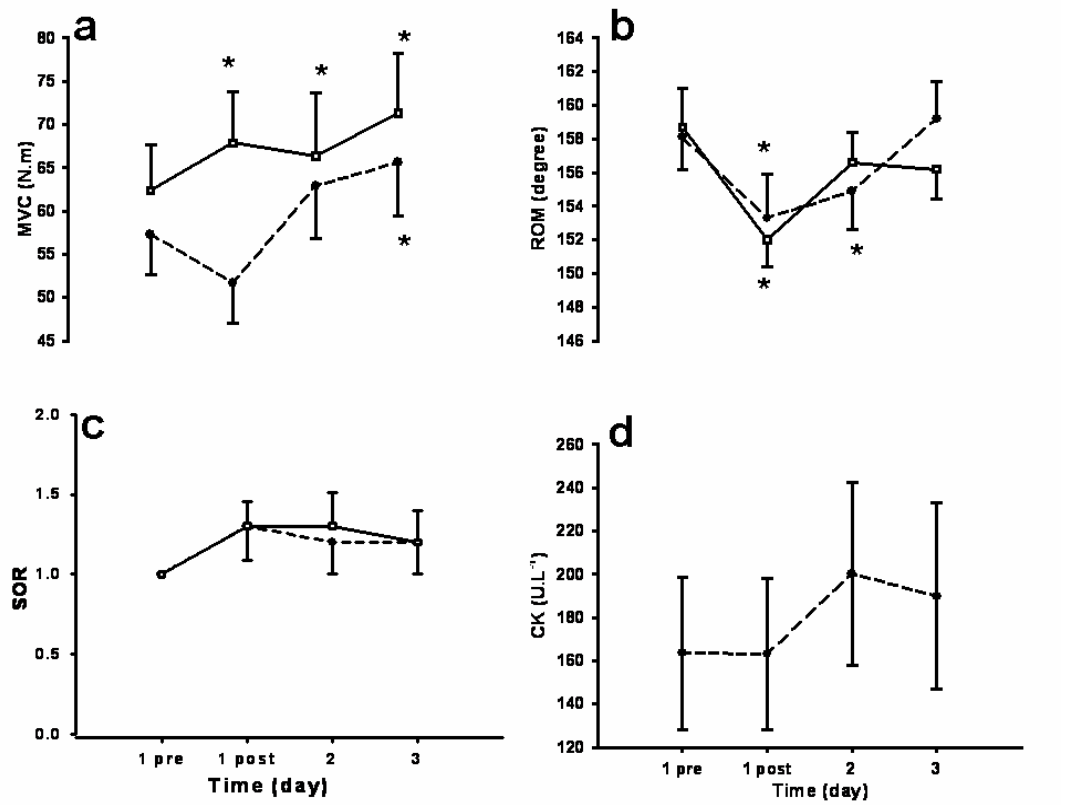

Figure 7: a) Maximum voluntary contraction (MVT), b) range of motion (ROM) at the elbow joint, c) subjective perception of muscle soreness (SOR), and d) Plasma Creatine Kinase activity (CK) before and after concentric contractions within Exercise (dashed line) and Control (solid line) arms. * denotes significant ($p<0.05$) differences between day 1 before concentric contractions and other days. Data are mean ± SE.

Resting O_2Sat, HbO_2 and mVO_2 increased at post-CE (18%, 21% and 52%, respectively), but had recovered within the next day (Figure 3-a, 3-b, and 3-d, respectively). In contrast, HHb decreased significantly (52%) at post-CE and recovered within the next day (Table 1). The changes in resting values were observed only on the Exercise limb. THb (Table 1) and mBF (Figure 3-c) did not change significantly, although there was a slight trend for increased (P=0.08) mBF.

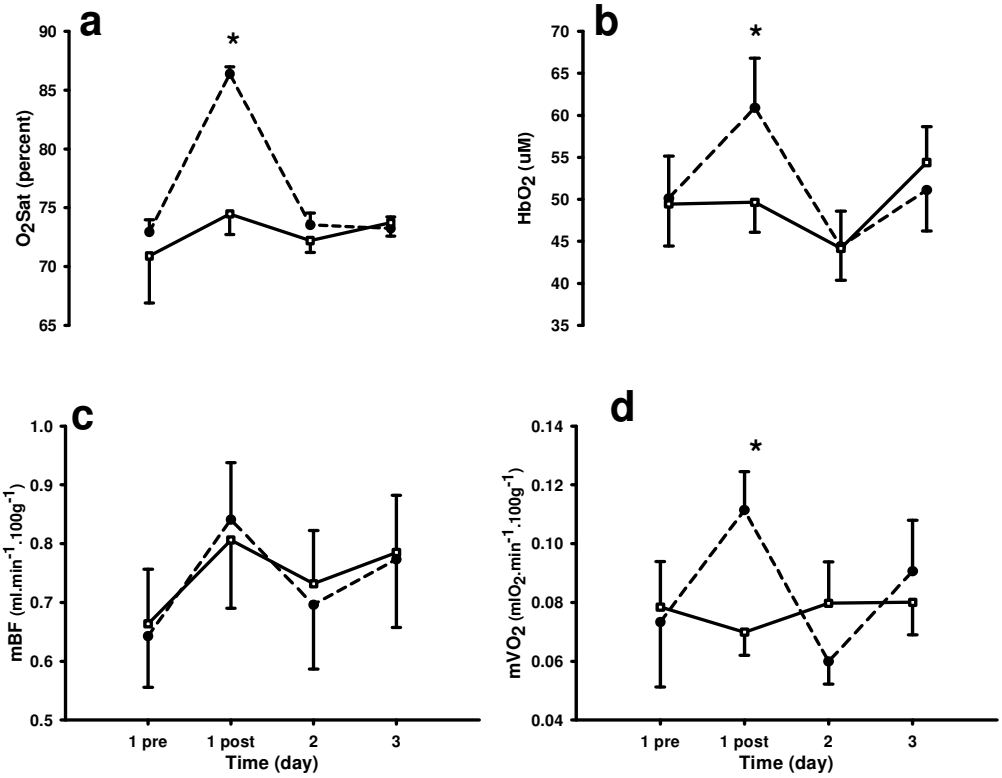

Figure 8: a) Resting oxygen saturation (O_2Sat), b) oxyhaemoglobin (HbO_2), c) muscle blood flow (mBF), and d) muscle oxygen consumption (mVO_2) before and after concentric contractions within Exercise (dashed line) and Control (solid line) limbs. * denotes significant ($p<0.05$) differences between day 1 before concentric contractions and other days. Data are mean ± SE.

There were significant arm-by-time interaction effects for ΔdeO_2Sat and Tau-deO_2Sat during arterial occlusions (Figure 4-a and 4-b, respectively). Subsequently, univariate ANOVA revealed that Tau-deO_2Sat and ΔdeO_2Sat had increased significantly at post-CE within Exercise and had recovered by day 2. There was not any arm-by-time interaction effect for ΔreO_2Sat or Tau-reO_2Sat (Figure 4-c and 4-d, respectively).

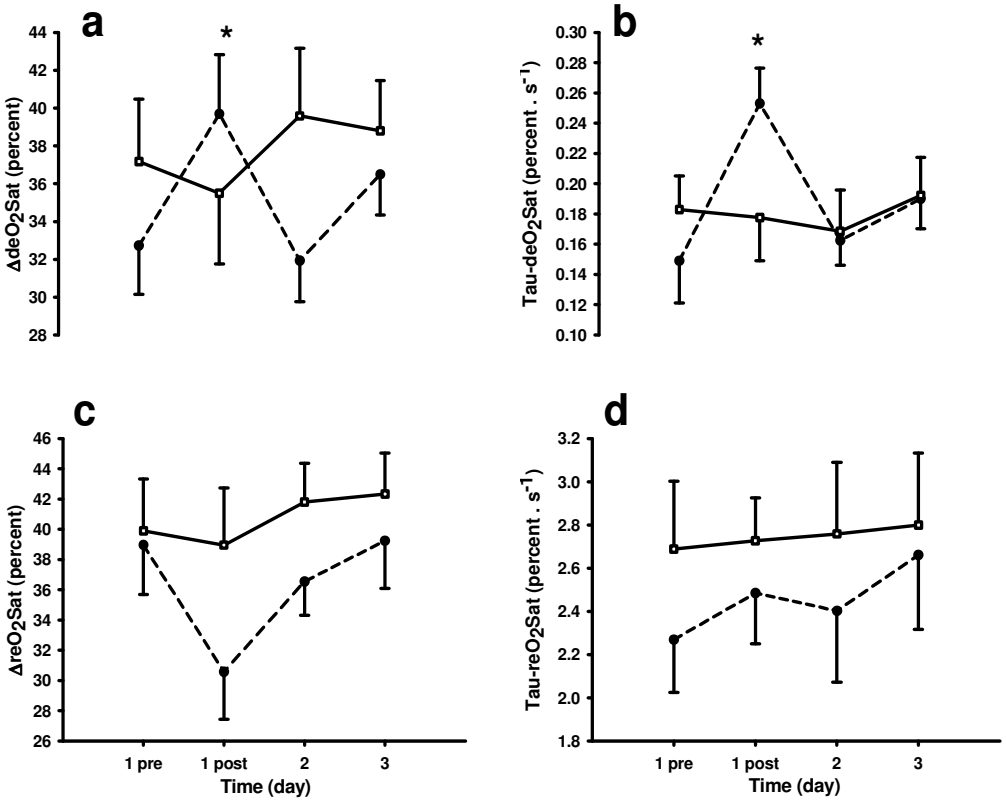

Figure 9: a) ΔdeO_2Sat, b) Tau-deO_2Sat, c) ΔreO_2Sat, and d) Tau-reO_2Sat during super-systolic occlusions before and after concentric contractions within Exercise (dashed line) and Control (solid line) limbs. Note: ΔdeO_2Sat (percent) refers to changes in muscle oxy gen desaturation, which were calculated as the difference in oxygen saturation from resting levels to nadir. Tau-deO_2Sat (percent • s^{-1}) refers to the rate of change in ΔdeO_2Sat from rest to nadir. ΔreO_2Sat (percent) refers to changes in muscle oxygen resaturation from nadir to peak recovery. Tau-reO_2Sat (percent•s^{-1}) refers to the rate of change in ΔreO_2Sat from nadir to peak recovery. * denotes significant ($p<0.05$) differences between day 1 before downhill walking and other days. Data are mean ± SE.

There were not any significant changes in NIRS-derived variables (i.e. ΔdeO_2Sat, Tau-deO_2Sat, ΔreO_2Sat and Tau-reO_2Sat) during IC30 (Table 1). In contrast, there were significant arm-by-time interaction effects for Tau-deO_2Sat and Tau-reO_2Sat during IC50 (Figure 5-b and 5-d, respectively). Consequently, univariate ANOVA showed a trend of decrease (9%) for Tau-reO_2Sat at post-CE, and a trend of increase towards day 3 within the exercise arm (the increase was statistically significant on day 3). Univariate ANOVA, however, did not show any significant change of Tau-deO_2Sat within either arm, although it followed a pattern similar to that of Tau-reO_2Sat (i.e. a decrease at post-CE and increases afterwards). No significant arm-by-time interaction effect was

observed for ΔdeO_2Sat and ΔreO_2Sat during IC50 (Figure5-a and 5-c, respectively). During IC80, there was a significant decrease in ΔreO_2Sat at post-CE within Exercise arm, which had recovered by day 2, and there were not any significant changes in ΔdeO_2Sat, Tau-deO_2Sat and Tau-reO_2Sat (Table 1).

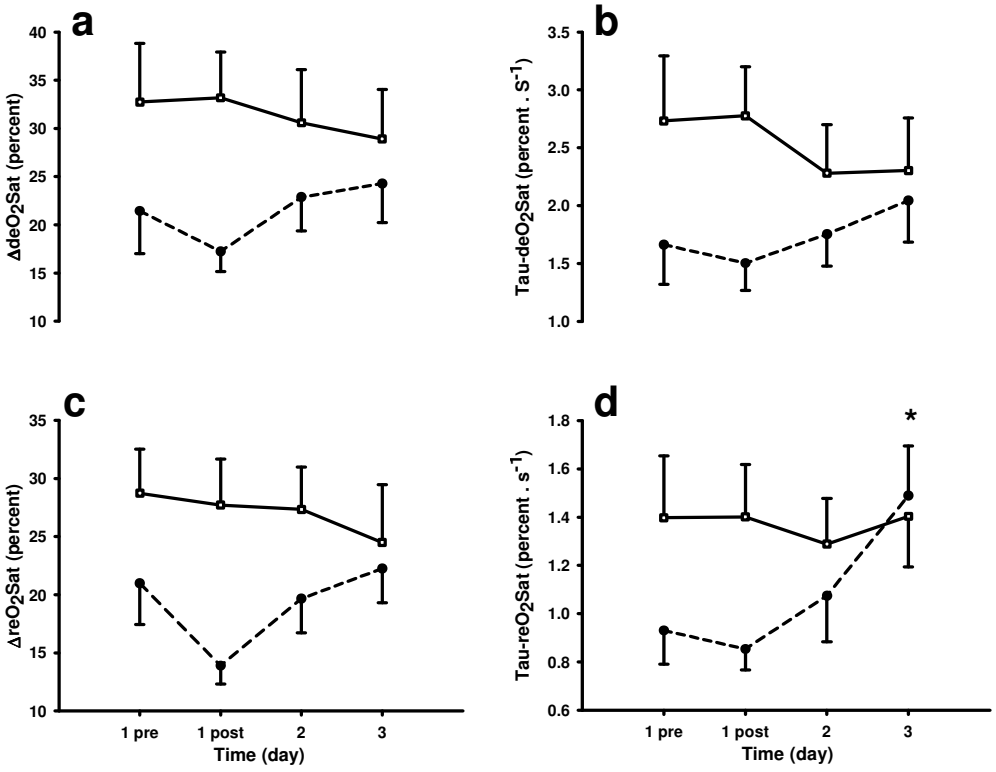

Figure 5: a) ΔdeO_2Sat, b) Tau-deO_2Sat, c) ΔreO_2Sat, and d) Tau-reO_2Sat during isometric contractions at 50% MVT, before and after concentric contractions within Exercise (dashed line) and Control (solid line) limbs. Note: All abbreviations are described in Figure 4. * denotes significant ($p<0.05$) differences between day 1 before downhill walking and other days. Data are mean ± SE.

6- Discussion

In this investigation, NIRS was employed to monitor muscle oxygenation of biceps brachii during rest and isometric exercises for two days following a heavy session of concentric exercise. We also investigated some of physical and metabolic characteristic of the muscle within Exercise and Control limbs after such exercise. The rational for this study was to produce study-study comparisons to previous finding of changes in physiological measures during arm and leg eccentric exercise (Ahmadi et al, in review).

Our findings revealed that MVT decreased by 10% at post-CE, but had fully recovered, and even marginally increased within the next 2 days (Figure 2-a). After a heavy session of exercise, depending on its severity, duration and type, different amounts of force loss are usually observed [22]. However, in most cases, if there is no muscle damage, the amount of maximum force returns back to the baseline values within the next several hours. Concentric exercise may result in a muscle force loss of 10–30% immediately after exercise [22], but this short-term reduction in MVT, which is usually recovered within 24 hr, is usually attributed to muscle fatigue within the Exercise limb.

The findings of this study showed that resting O_2sat and HbO_2 increased and HHb decreased after CE and recovered within the next day. Assuming that O_2Sat reflects the dynamic balance between O_2 supply and O_2 consumption [23], an acute increase in O_2sat after exercise may be required for EPOC. This hypothesis was supported by the increase in the resting mVO_2 at post-CE. mVO_2 could remain high for up to few hours after exercise depending on the exercise type, duration and intensity, as well as the individuals' training state [10].

However, mBF did not change significantly after CE, although there was a trend to an increase (P= 0.08) for this variable after exercise. mBF might have recovered within the 30-min recovery period when the post-CE assessments were performed. A previous study has suggested that mBF response in recovery is dependent on type, duration and tension of exercise, and it could remain high for up to 30-min after exercise [24]. Nevertheless, there has been some evidence suggesting that blood flow has not been regulated through a mechanism sensing the need for oxygen by the exercised muscle [24]. Therefore, the mBF response to exercise could be different from that of mVO_2.

During arterial occlusions, there were significant increases in oxygen desaturation volume and kinetics (i.e. ΔdeO_2Sat and Tau-deO_2Sat, respectively) at post-CE. This finding might indicate that in a resting ischemic condition (e.g. where there is no blood flow to the Exercise limb), the exercised muscle would consume the existing oxygen faster and to a larger amount compared to a Control contralateral limb. However, this

was not the case for oxygen resaturation volume and kinetics, which did not change after exercise.

Our findings showed a consistent pattern of changes in the NIRS-derived variables measured during isometric contractions of different intensities. As is shown in Figure 5 (a-d) and Table 1, there was a trend to a decrease in ΔdeO_2Sat, Tau-deO_2Sat, ΔreO_2Sat and Tau-reO_2Sat at post-CE, which had recovered on day 2 and slightly increased by day 3. Although the changes in NIRS-derived variables during isometric contractions were not significant (except for in Tau-deO_2Sat and Tau-reO_2Sat during IC50, and ΔreO_2Sat during IC80), the consistency of these changes may indicate the need for a further investigation.

Interestingly, the pattern of changes in muscle oxygenation during isometric contractions was similar to the changes in maximum force during MVTs (Figure 2-a). Collectively, these findings might indicate a lesser and slower oxygen desaturation and resaturation time-course during isometric contractions in the Exercise arm after CE. A decrease in oxygen desaturation kinetics could be due to insufficient blood flow (and consequently O_2 delivery) to the muscle, and/or the muscles inability to extract oxygen from capillaries. After a heavy session of exercise, muscle blood flow has generally increased [24], possibly to provide the oxygen required for EPOC and to remove the metabolites augmented during exercise. On the other hand, the intramuscular pressure and the water content of muscle might also increase during repetitive contraction, which in turn could result in muscle swelling and inflammation. Although this inflammation might not be an obstacle for blood flow during rest, it could decrease O_2 diffusion from capillary to muscle fibre during isometric contractions, where there were extra pressures on vessels due to contraction. In this circumstance, a decrease in O_2 availability and O_2 uptake could result in a decrease in O_2 desaturation during exercise, despite the presence of an increased blood flow. A decrease in oxygen desaturation kinetics could also be due to the muscle insufficiency to extract O_2 from capillaries. Exercise could be associated with perturbations of the intracellular milieu, which could alter mitochondrial function [25]. Mitochondrial oxidative stress resulting from excess reactive oxygen

species along with other factors such as temperature, catecholamines, creatine kinase etc. might slow the oxygen desaturation kinetics during isometric contractions.
One may speculate how could the biceps brachii muscle produce the same amount of force (e.g. 50% of MVT) before and after CE, while it was taking and, therefore, consuming less oxygen after CE? Although there is not a certain explanation, it could be because of a different recruitment of muscle units and/or fibres compared to the pre-CE condition. For example, the muscle might have recruited more fast twitch fibres after the CE session, which might require and consume less oxygen. Moreover, there are some other elbow flexor muscles that have major contributions in elbow flexion. Movement synergists frequently alter functional responsibilities. Therefore, it is possible that due to fatigue of biceps brachii after CE, other elbow flexors have made greater contributions to isometric elbow flexions compared to the unfatigued state (before CE). This might have reduced the contribution of biceps brachii for force production, and therefore, it might decrease the amount of muscle oxygen consumption.

In conclusion our findings showed some significant changes in muscle oxygenation during rest and exercise after a session of maximal concentric contractions, which recovered within the same the day after exercise. Therefore, it can be concluded that concentric exercise did not produce prolonged changes in muscle oxygenation, and the observed findings might reflect the excess post exercise oxygen consumption. We also observed some non-significant but consistent trends of changes in oxygen desaturation and resaturation volume and kinetics during isometric contractions, which lasted for 2 days and were in a similar pattern to that of force during MVT. Further investigations with larger sample sizes could better reveal the existence of such a pattern, if there is any, and explore the underlying mechanism(s).

7-Study limitations

In this study, we employed the non-dominant arm as the Exercise, and the contralateral limb as the Control. There is a possibility that the contralateral elbow flexors had been activated during concentric contractions of the Exercise limb. We did not monitor muscle oxygenation or the muscular activity of the Control limb during CE, and this

might have had a confounding effect on our findings. Moreover, because of its anatomical position, we monitored biceps brachii as the only elbow flexor in this study. There are some other elbow flexors that take part in elbow flexion. For example, as compared to biceps brachii, the brachialis muscle has a larger cross sectional area and is the prime elbow flexor. Therefore, it is possible that the functional responsibilities of the elbow flexors have altered during isometric contractions performed in this study, and this might have a confounding effect on our findings.

8- Acknowledgment

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Chapter 7

Discussion and Conclusions

Introduction

Oxygen delivery and utilization are vital for the normal function of the musculoskeletal system. The quantity and quality of muscle oxygenation and myoelectrical activity could affect and/or be affected by exercise-induced muscle damage. This was the primary rationale for this dissertation. Three studies were carried out to quantify the effects of different types of heavy exercises on muscle oxygenation, using a noninvasive technique (i.e. NIRS):

- Experiment 1 monitored muscle oxygenation (Chapter 3) and electromyographic activity (Chapter 4) within biceps brachii muscle following vigorous eccentric arm contractions,
- Experiment 2 evaluated muscle oxygenation within vastus lateralis following prolonged downhill walking exercise (Chapter 5), and,
- Experiment 3 monitored muscle oxygenation within biceps brachii following concentric arm contractions (Chapter 6).

It was hypothesized that muscle oxygenation, blood flow, and myoelectrial activity within the exercised muscle would change for several days after eccentric, but not concentric exercise. As an adjunct to investigating muscle oxygenation, some mechanical and biochemical characteristics of muscle were assessed within each study.

Issues that are relevant to specific observations have been presented in the Chapter accompanying each study. Therefore, this Chapter conceptually summarizes the outcomes of the three experiments within this dissertation. In addition, the limitations of the studies performed in this manuscript and future research directions are discussed. For simplicity, the experimental studies are referred to herein as Chapters 3, 4, 5 and 6.

Physical, biochemical and myoelectric characteristics of exercised muscles

During exercise, numerous alterations occur in the body organ systems, but specifically within the exercising muscles. The quality and quantity of these changes varies

depending on the type, duration and intensity of the exercise protocol, as well as the individual's training state. Investigating possible alterations of muscle oxygenation was central to this work, and as previously described, three different exercise protocols were carried out to test the hypotheses.

Muscle strength

Maximal muscle force was isometrically measured in all experiments and referred to as maximal voluntary isometric torque (MVT) in all chapters. Collectively, muscle maximal strength decreased immediately and significantly after both concentric and eccentric exercise stressors, although the magnitude of the decrements was not equivalent. MVT recovered by the next day after concentric exercise, while it did not return to the baseline values for several days after the eccentric protocols. The changes in organization of the sarcomere structure and changes in excitation-contraction coupling after eccentric exercise could be the main contributors to the force loss (Morgan and Allen 1999).

Although the possible reason(s) for the differences in force loss observed after eccentric vs. concentric exercise is not yet known, the following assumptions may apply. Eccentric exercise causes more muscle damage than concentric contractions. Greater damage is primarily due to a mechanical insult rather than metabolic fatigue. This is possibly due to the fact that, as muscle lengthens, the ability to produce tension increases and a higher load is distributed amongst the same number of fibres, and therefore, results in a higher load per fibre ratio (Clarkson and Hubal 2002).

The neuromuscular differences between eccentric and concentric contractions could predispose muscles undertaking eccentric contractions to greater degrees of strain and damage after exercise. These differences include a decreased motor unit firing rate when switching from concentric to eccentric contractions, increased motor unit synchronization during lengthening vs. concentric contractions, and increased variability in the modulation of activity of motor units (i.e., discharge rate and patterns) during eccentric vs. concentric exercise (Clarkson and Hubal 2002).

Prolonged force loss over several days, especially after eccentric exercise, is one of the most valid and reliable indirect measures of muscle damage in humans (Warren et al. 1999). Therefore, it was noted that our EE protocols were effective to elicit damage in arm or leg muscles. In contrast, the temporary reduction of force that transpired after concentric exercise could be simply due to post-exercise fatigue, which usually recovers within the next few hours after exercise. These findings were in line with the previous studies with similar protocols (Nosaka and Clarkson 1994; Sayers et al. 2000; Farr et al. 2002).

Muscle soreness

Soreness is a common finding congruent with muscle damage, which generally starts after 24 hr, peaks between 24 to 48 hr and decreases by 5-7 days after the exercise event (Clarkson and Hubal 2002). In Chapters 3, 4 and 5, significant levels of muscle soreness were reported by subjects after eccentric exercise (EE) protocols, although their duration and magnitude were less after downhill walking compared to biceps brachii EE. Soreness was not a reported observation after concentric contractions in Chapter 6. As was explained earlier, there is a greater chance of muscle damage being produced after eccentric vs. concentric contractions, and this might explain why soreness was not reported after concentric exercise.

It is possible that soreness results from swelling and pressure in the muscle (Clarkson et al. 1992; Clarkson and Hubal 2002). The inflammatory responses after high-force eccentric exercise results in the accumulation of neutrophils and macrophages at the site of tissue injury (Smith 1991), and consequently evoke sensation of muscle soreness (Sayers et al. 2000). Proske and Allen (2005) have suggested that muscle soreness could be an allodynia, in which changes of neural processing at the level of the spinal cord allow mechanoreceptors, to access the pain pathway. However, it should be noted that although soreness has been widely measured in literature as an indirect maker of muscle damage, it has correlated poorly with changes in muscle function, both in terms of magnitude and time-course (Warren et al. 1999).

Serum creatine kinase (CK) activity and myoglobin (Mb) concentration

Blood levels of CK and Mb have been used as markers of eccentric contraction–induced injury. Under normal conditions, CK is located within muscle fibres and does not diffuse outwards. However, when exercise is intense and a cell is injured, CK is released into the blood stream where it can be detected via biochemical analyses (Lieber 2002). Therefore, CK serves as an indirect marker of myofibrillar integrity and injury (Lieber 2002). As reported in Chapter 3, CK activity increased significantly after EE, peaked on day 5 and remained higher than the initial value until day 7 in most subjects, and rose even further by day 7 in some individuals. The changes in CK reported in Chapter 5 were not as great as those within the Chapter 3. In addition, CK did not change after concentric exercise in Chapter 6. This was predicted because no muscular damage was expected after concentric exercise.

However, it is not clear why the two types of exercise predominantly used to study muscle damage (i.e., downhill running and high-force muscular eccentric contractions), show very different CK responses (Clarkson and Hubal 2002). It has been often suggested that the level of CK could by some means be related to the magnitude of muscle damage, although this idea has not been examined properly (Lieber 2002). If this correlation exists, it might be suggested that the level of muscle damage was less in Chapter 5 compared to Chapter 3. This could be due to the relatively lower exercise intensity during downhill walking compared to eccentric contractions of biceps brachii. The variability of changes in CK activity and the possible reasons for these changes have been discussed by other authors (e.g. Clarkson et al. 1992; Schwane et al. 2000). Nevertheless, the large variability in CK response to exercise is not understood and does not seem to be related to sex, muscle mass, or activity level of the subjects (Clarkson and Hubal 2002). Even after similar types and intensities of exercise, a large inter-subject variability has been reported (Newham et al. 1983).

In addition to CK activity measurements in all experiments, Mb concentrations were measured in Chapters 5 and 6, in order to further investigate the biochemical changes in muscle and blood after eccentric or concentric efforts. Myoglobin is found primarily in cardiac and red skeletal muscles, functions in the storage of oxygen and facilitates the transport of oxygen to the mitochondria for oxidative phosphorylation. This allows the exercising muscle to maintain a high level of activity for a longer period of time. When muscle is damaged, Mb is released into the bloodstream in a similar pathway to that of CK. Therefore, an increase in serum myoglobin could reflect the presence of muscular and/or cardiac damage. Mb increased significantly after downhill walking and, although it recovered on day 3, it was again significantly higher than the baseline value on day 5 (Figure 5; Chapter 3). No muscular damage was observed after concentric exercise in Chapter 6 and accordingly no alteration was found in serum Mb values.

However, the use of CK, Mb and any other muscle protein measures to distinguish and categorize muscle damage should be considered with caution for the following reasons. First, a muscle fibre's permeability to intramuscular enzymes might or might not be correlated with cellular contractile function (Lieber 2002), and second, blood concentration is a function of what is being both produced in the muscle and cleared from the blood (Warren et al. 1999; Clarkson and Hubal 2002). In other words, blood levels of myofibre proteins reflect not only their release into the blood but also their removal. Alterations in function of, and/or blood flow to, the tissues that involve in clearing these proteins affect the blood levels of myofibre proteins, as does exercise-induced haemo-concentration or hemodilution (Warren et al. 1999). Finally, the large inter-individual variability of responses to exercise is perhaps the biggest problem with the application of muscle proteins for the detection of muscle damage (Clarkson and Hubal 2002).

Limb circumference

Prolonged swelling is a common symptom of muscle damage. The increases in circumference, muscle thickness, and muscle area seem to be related to inflammatory swelling (Nosaka and Clarkson 1996). Accumulation of fluid in the damaged area result

in swelling, and when fluid accumulation exceeds capability of lymphatic drainage, edema is produced (Guyton 1986). In order to assess the existence of swelling and/or edema, in all experiments presented in this dissertation, limb circumference was measured at various anthropometrical locations on and around exercised muscles.

As described in Chapter 3, CIR increased within 24 hr after EE at all anthropometrical locations (i.e., 4, 6, 8, and 10 cm above elbow), and did not recover for the next 5 days. This is in agreement with the previous similar studies and supported the effectiveness of the experimental protocol to evoke muscle damage (Clarkson et al. 1992; Felici et al. 1997). In a careful study using magnetic resonance imaging and ultrasound techniques, Nosaka and Clarkson (1996) suggested that most of the fluid accumulation in the upper arm was in the endomysium of muscle fibres or in the intracellular space of the fibres, and remained there at least 5 days after exercise.

Contrary to the findings of Chapter 3, CIR at all three anthropometric locations (6, 10, and 14 cm above knee) revealed no changes within and between experimental days after downhill walking. This could be due to the following reasons. Thigh circumference includes several muscles and big layers of adipose tissue that might have not been affected (damaged) by downhill walking. Therefore, the swelling in quadriceps muscle due to downhill exercise, if any, might not be detected by measuring the whole thigh circumference. Additionally, the magnitude of damage could affect the amount of swelling. As supported by other markers of muscle damage, the magnitude of damage after downhill exercise in Chapter 5 was less than that of Chapter 3.

Two way repeated measurements in Chapter 6 revealed no time (3 days) by arm (Control vs. Exercise) interaction effect for CIR at all locations (i.e., 4, 6, 8 and 10 cm above elbow). As described earlier, muscle swelling is expected after damage inducing exercise. Chapter 6 revealed no evidence of muscle damage within the exercised arm; therefore, swelling was not expected. However, there was a trend of day-day increase at some locations for both arms. These minor changes could be due to the minor increase in intramuscular pressure and water content of the muscle after isometric contractions at different intensities.

Range of Motion

ROM is defined as the arc over which a joint may operate, and this constrains the muscle length range. In addition to the muscle characteristics, ROM is determined by skin, subcutaneous tissue, tendon, articular capsule and bone properties (Warren et al. 1999). Changes in the property of connective tissue or changes in tendon at its attachment are the possible reasons for a decrease in the muscle ability to extend to its pre-damage condition (Howell et al. 1985; Newham 1988; Clarkson et al. 1992). On the other hand, the inability of stretched sarcomeres to produce maximal sliding together of the actin/myosin filaments could explain the increase in elbow flexion angle after EE (Clarkson et al. 1992). Together, an increase in flexion angle and a decrease in extension angle would result in a decreased range of motion after exercise induced muscle damage.

In this thesis, ROM was assessed after both concentric and eccentric contractions of elbow flexors. The findings of Chapter 3 revealed that ROM decreased and did not recover for several days after eccentric exercise. This further showed that the EE protocol employed in chapters 3 and 4 has been efficient to evoke muscle damage. Results of Chapter 6 did not reveal any significant change in ROM. This was an expected outcome, because concentric exercise did not produce muscle damage as supported by other measures.

Muscle myoelectric activity

The analysis of the surface electromyogram (EMG) signal has been used to detect changes in the myoelectric behaviour of a muscle both during and after EE (Berry et al. 1990; Felici et al. 1997; McHugh et al. 2000). However, there has been a wide range of differing outcomes and viewpoints amongst groups who have studied the effect(s) of EE on EMG. In Chapter 4, authors attempted to clarify the effect of EE on the electrical behaviour of biceps brachii during isometric contractions (i.e. 50% and 80% of MVT), deriving root mean square (RMS) and median frequency (MDF) as EMG parameters. Linear regression of the RMS and MDF time-series (20 s sustained IC50 and IC80) was

used to extract intercepts and slopes of these signals on each day, and reduced a plethora of time-epoch data to simpler regression parameters of slope (β_0) and intercept (β_1) during each muscle contraction. Changes of these intercepts and slopes across days were proposed to describe possible changes of EMG over time after exercise-induced muscle damage.

The results of the EMG data revealed that MDF linear regression intercept decreased significantly after acute EE during both 50% (IC50) and 80% (IC80) MVT within the Exercise arm and remained significantly less than the baseline value on day 3 during IC50 (Figure 4; chapter 4). Although MDF intercept recovered within the next two days after EE, there was a general trend to a lower than baseline value until day 6 for this variable. These findings were in agreement with some studies (Felici et al. 1997; Day et al. 1998), but not in line with the results of others (Komi and Viitasalo 1977; Berry et al. 1990; McHugh et al. 2000).

Muscle fibre conduction velocity that could be reflected by MDF is higher for fast-twitch compared to slow-twitch fibres (Andearssen and Arendt-Nielsen 1987). This means that the more fast-twitch fibres involved in a contraction, the higher MDF. Fast-twitch fibres are more susceptible to damage and fatigue (Berry et al. 1990), and consequently a shift towards greater recruitment of slow-twitch motor units may decrease stress on the susceptible fast-twitch fibres (McHugh et al. 2001). Therefore, a decrease in MDF intercepts could be the result of a preferential reduction in the recruitment of fast-twitch fibres. Additionally, the changes in intra muscular pressure, as well as the changes in water content and blood volume of the muscle might alter the EMG findings. Blood flow can affect the characteristics of the surface-recorded signal by imposing a low-pass filter medium. This tissue filtering can decrease the frequency content of the signal (Kamen and Caldwell, 1996). On the other hand, an increase in blood flow generally increases local temperature, which can change spectral features of the EMG signals (Holewijn and Heus, 1992).

After EE in Chapter 4, MDF decreased over time during sustained isometric contractions (Figure 1; Chapter 4). These decrements (MDF slopes), were observed in

both Control and Exercise arms and at both intensities (IC50 and IC80). However, there were no significant day-to-day changes amongst the slopes obtained from different arms and different intensities (Table 1; Chapter 4). This suggests that in a sustained situation such as a 20-s isometric contraction, the rate of decrease in MDF was independent of EE-induced muscle damage. This result was not in agreement with those of Kroon et al. (1991), who observed an increase in the slope of mean power frequency after EE. The methodological differences in collecting and analysing EMG data, as well as in eccentric exercise protocols, could be the main reasons for these dissimilar findings.

A possible mechanism for the decrease in MDF during prolonged contractions is the external accumulation of potassium ions (Mills and Edwards 1984). An outward leakage of potassium resulting in an ionic imbalance around sarcolemma might slow the action potential and consequently decrease MDF (Day et al. 1998). This explains the decrease in MDF (MDF slope) during sustained contractions in both Control and Exercise arms.

The findings of our study did not show any consistent alteration in RMS values (Table 1; Chapter 4). These results supported the findings of other authors who either did not find any significant changes in RMS after EE (Sayers et al. 2001), or their RMS data was not statistically linear, and they did not perform linear regression analyses on RMS values (Felici et al. 1997). However, our findings were in contrast to those of Berry et al. (Berry et al. 1990). RMS is a time domain parameter and, it has been reported that RMS is more susceptible to the day to day changes compared to MDF (Merletti et al. 1995; Felici et al. 1997). In other words, RMS may change over days regardless of muscle fatigue or soreness. Therefore, it could be rationally expected that there would be inconsistency in RMS values over time.

Overall, the EMG analyses of day-day changes of MDF or RMS was less promising than had originally been anticipated. The results reported herein were somewhat inconsistent amongst days or incongruent to some previous studies. Such inconsistency could not be due to low statistical power, as the linear regression approach reduced the data from 10 subjects with 16 time-series measure per individual over 7 days to measure

linear regression coefficients (β_0, β_1)- a standardized approach (Merletti et al. 1995; Felici et al. 1997). It is possible that these EMG data have little utility in describing post exercise muscle damage. For these reasons, this approach was abandoned in the downhill walking and concentric exercise experiments.

Muscle oxygenation and blood flow measured by NIRS

For the purpose of this dissertation, muscle oxygenation was the main investigated variable in all experiments. Understanding of mechanisms and/or factors that control muscle oxygenation immediately after exercise may help to explain the possible long-term effects of exercise on muscle oxygenation, if any. Therefore, the following section first introduces and describes some factors affecting muscle oxygenation and then discusses the findings of each Chapter.

Muscle oxygen consumption (mVO_2) could remain high for up to few hours after exercise depending on the exercise type, duration and intensity, as well as the individuals' training state. The term excess post-exercise oxygen consumption (EPOC), which was introduced by Gaesser and Brooks (1984), refers to the oxygen consumption above resting requirements after the cessation of exercise. This elevated post-exercise metabolism contributes to the energy cost of exercise and hence the thermic effect of activity (Laforgia et al. 2006). In this thesis, EPOC was not directly measured, instead mVO_2, as one of the main components of EPOC, was quantified approximately 30-min after exercise treatment in all experiments, using NIRS.

Lactic acid had been classically assumed as the main determinant of EPOC (Hill and Lupton 1923). However, creatine phosphate (CP), catecholamine, creatine kinase, calcium^{2+} and temperature have also demonstrated significant effects on post-exercise mVO_2, according to the current literature (Gaesser and Brooks 1984; Laforgia et al. 2006). In fact, EPOC is the result of a general metabolic perturbation, and the repayment of the oxygen deficit may only partially contribute to it (Laforgia et al. 2006). It has been reported that phosphagen restoration and lactate metabolism together with elevated body temperature contributes to only about 30 – 50% of the 1 hr EPOC

following submaximal and supramaximal work (Bahr et al. 1992). After 1 hr, it seems that EPOC is primarily associated with triacylglycerol/fatty acid cycling (Bahr et al. 1990; Laforgia et al. 2006).

Since the mitochondrion is the site of oxygen consumption in the cell, the rationale for EPOC could be found at the level of this cellular organelle. Mitochondrial function could be controlled directly by concentrations of ADP, ATP, Pi, and CP. Although post-exercise mVO_2 will likely be controlled directly by intra-mitochondrial ADP level, kinetics of the post-exercise mVO_2 phenomenon is influenced by a variety of factors such as catecholamine, thyroxin, glucocorticoids, fatty acids, calcium ions, and temperature (Gaesser and Brooks 1984). Some of the factors, which indirectly control mitochondrial respiration have been illustrated in Figure 1, and have been well explained and reviewed by Gaesser and Brooks (1984) as follow:

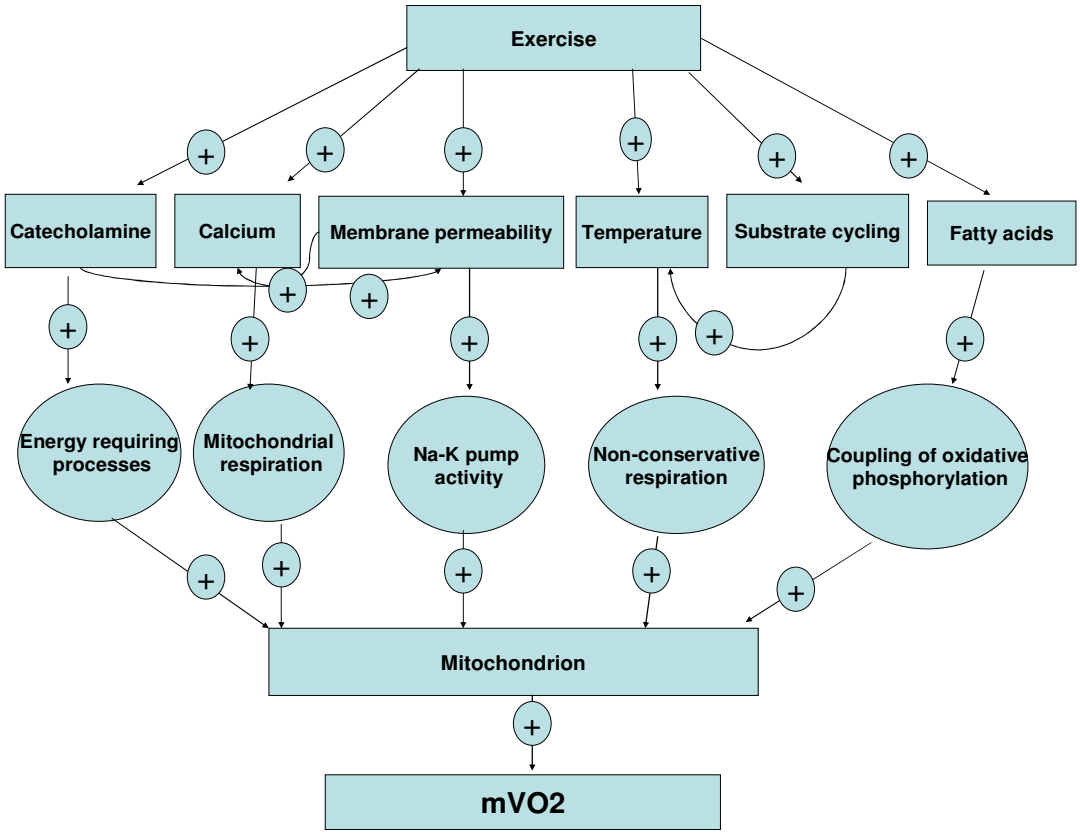

Figure 1. Some factors that could control mitochondrial respiration indirectly. + refers to a positive (stimulating) effect.

Catecholamines could elevate mitochondrial respiration by stimulating energy-requiring processes in the cell. They also increase the cell membrane permeability to sodium (Na^+) and Potassium (K^+) ions. The increase in Na^+-K^+ pump activity would require an increase in ATP production and, therefore, oxygen consumption.

Calcium ion might affect the post-exercise mVO_2 by stimulating mitochondrial respiration. Increased amounts of calcium within mitochondria could also affect the linkage between oxidation and phosphorylation, which could consequently result in an increased rate of oxygen consumption.

Temperature could be the most important factor controlling EPOC. The extent to which temperature affects the post-exercise mVO_2 could be related to the effects of elevated temperature on mitochondrial energetics. Elevated temperatures could increase non-conservative (stage 4) respiration, which in turn would increase mVO_2 for the synthesize of a given amount of ATP.

Substrate cycling could be the cause of the elevated temperature observed for several hours after exercise. *Fatty acids* also may influence the coupling of oxidative phosphorylation in mitochondria and affect post exercise mVO_2.

In addition to the above factors that control mitochondrial respiration both directly and indirectly, some other factors affect oxygen delivery and consumption within the exercised muscle. For example, an increased oxygen consumption and metabolic stress during prolonged exercise may increase the production of reactive oxygen species (ROS), which may affect mitochondrial function (Tonkonogi et al. 1998). Additionally, increased water content of muscle and increased intramuscular pressures (Friden et al. 1983) may change the pattern of local blood flow and muscle oxygenation. Furthermore, during exercise, the vascular portion of active muscles is considerably increased by the dilation of local arterioles (Mcardle et al. 1986). Therefore, an increase in mBF during and immediately after any type of exercise could be expected. The skeletal mBF is closely related to muscle oxygen uptake during exercise (Andersen and Saltin 1985), hence, an increase in mBF could result in a rise in mVO_2.

The model presented in Figure 2 details some of the many contributing factors that might affect post exercise oxygen consumption and the interactions between these factors. The relative contribution of each of these factors might be influenced by variables such as exercise intensity and duration, individuals' state of training and environment. In experiments presented in this thesis, possible effects of external variables were minimize by recruiting subjects with similar training background and performing similar exercise durations and intensities in a similar environmental condition.

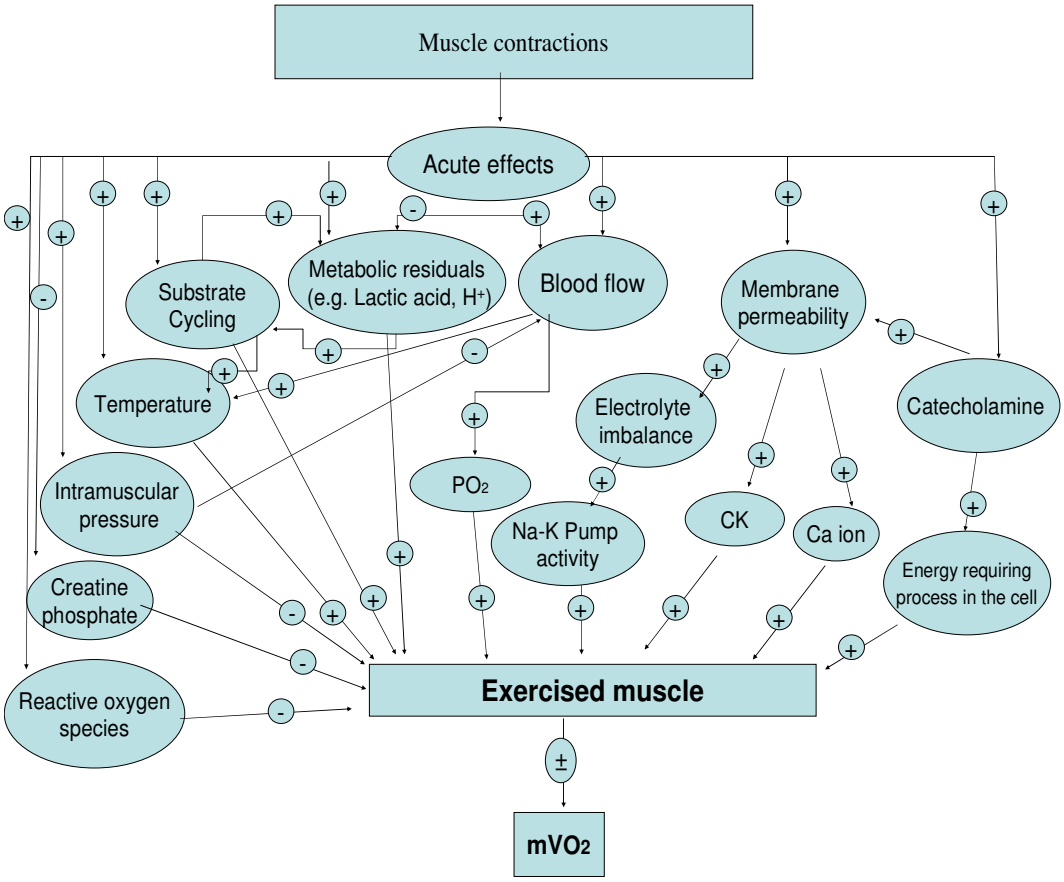

Figure 2: A proposed model for some of the factors affecting muscle oxygen consumption (mVO_2) after exercise. Arrows refer to the direction of effect, + refers to positive (stimulating) effect, – refers to negative (inhibiting) effect, and ± refers to the possibility of a positive or a negative effect.

Because of the dissimilarity between the methodologies, especially between Chapters 3 and 5, the outcomes of all Chapters could not be compared precisely. Nevertheless, in

this section, the experimental findings of this dissertation were compared by presenting the percentage of changes in each variable rather than its absolute value. It should also be noted that the data presented in this section is mostly from exercise limbs; however, the findings reported from Chapter 5, are the average of the outcomes from both limbs, as there was no control limb. For a better comparison and understanding of the outcomes, the NIRS-measured variables in all experiments have been divided and explained in two categories, indicating acute and chronic responses to exercise.

Acute effects of exercise on muscle oxygenation

Post-exercise responses

The results of Chapter 3 demonstrated that 30-min after eccentric exercise, recovery mBF and mVO_2 had increased by 110% and 94%, respectively (Figure 3; Chapter 3). Similar findings were observed in Chapters 5 and 6, although the magnitudes of the post-exertion changes were dissimilar amongst experiments. For instance, in Chapter 5 post-exercise mBF and mVO_2 had increased by 39% and 30% (Figure 3; Chapter 5), but they increased by only 24% and 13%, respectively, in Chapter 6. These values reflected the percentage of mVO_2 30-min after each exercise protocol, which could be an indication of EPOC as was explained and assumed earlier in this Chapter. Energy turnover in skeletal muscle could increase by 400 times, and muscle oxygen consumption might increase by more than 100 times during exercise compared to the resting muscle (Tonkonogi and Sahlin 2002). Such increments, undoubtedly, require a very high and prolonged EPOC.

Post-exercise, tissue oxygen saturation (O_2Sat) increased by 21%, 1% and 18% after exercise, in Chapters 3, 5 and 6, respectively. The reason for the dissimilarity between the findings of Chapter 5 and other Chapters is unclear. However, some of the possible explanations for this discrepancy will be discussed further in this Chapter. Generally, O_2Sat reflects the dynamic balance between oxygen supply and consumption (Ferrari et al. 2004). An increase in O_2Sat, therefore, could reflect a higher supply of oxygen to the exercised limb and/or a possible decline in oxygen extraction due to mitochondrial disturbances. The concomitant increase in mBF, observed in all experiments, strongly

points to additional oxygen supply 30-min after exercise. Contrariwise, the possibility of a decrease in oxygen uptake was not supported by our findings because of the marked increase in mVO_2, observed after all exercise protocols.

In Chapters 5 and 6, oxygen desaturation and resaturation patterns were monitored during super-systolic arterial occlusions 30-min following eccentric or concentric exercises. This method has been employed in previous studies, to normalize NIRS-obtained data and to estimate immediate mVO_2 (e.g. De Blasi, 1991, Hamaoka, 2000, van Beekvelt, 2001 and Kooijman, 1997). In Chapter 5, during arterial occlusions after EE, the rate (Tau-deO_2Sat) and the amount (ΔdeO_2Sat) of oxygen desaturation increased by 38%, and 6%, respectively, after downhill walking. Tau-deO_2Sat and ΔdeO_2Sat increased by 21% and 64%, respectively after concentric exercise in Chapter 6. Taken together, these acute changes suggested that when there was no blood flow to the exercised muscles, these muscles became desaturated faster and to a greater degree than under pre-exercise conditions. In other words, the 30-min after exertion exercised muscles used more oxygen (i.e. higher mVO_2) in a shorter time compared to resting conditions. As previously noted, this could be explained by the same mechanisms that contribute to EPOC (Figure 2).

Exercise responses

During exercise, depending on the work intensity, the degree of activation of a particular muscle group and its trained state, skeletal muscles deoxygenate to varying degrees (Hampson and Piantadosi 1988; Boushel et al. 1998; Nioka et al. 1998). In this dissertation, the changes of muscle oxygenation (reflected by O_2Sat), was assessed during isometric contractions of different intensities (i.e. IC30, IC50, and IC80) approximately 50-min after vigorous concentric and eccentric exercise. These isometric assessments could further reveal any difference(s), between Exercise and Control limbs, as well as between pre- and post-exercise conditions.

Chapter 3 revealed that ΔdeO_2Sat, ΔreO_2Sat, Tau-deO_2Sat and Tau-reO_2Sat, which were assessed during isometric contractions post-exercise, decreased significantly after

EE at both IC50 and IC80 (Figure 4 and 5 in chapter 3). In contrast, all NIRS-obtained variables including ΔdeO_2Sat, ΔreO_2Sat, Tau-deO_2Sat and Tau-reO_2Sat that were measured during isometric contractions of different intensities, increased significantly after downhill walking except for ΔreO_2Sat during IC50 and Tau-reO_2Sat during IC80 (Figure 5 and Table1; Chapter 3). Furthermore, the findings of Chapter 6 showed a trend of decrease in those variables after acute concentric exercise, although these changes were not significant under most conditions (except for in Tau-deO_2Sat and Tau-reO_2Sat during IC50, and ΔreO_2Sat during IC80; Figure 1 and Table 1; Chapter 6).

It will be noted that the results of Chapter 5 were in contrast to those of Chapters 3 and 6. This disparity made it difficult to interpret our findings, although some of the possible contributors to this divergence will be discussed further within this chapter. One may logically expect that during isometric contractions after a session of vigorous exercise, oxygen desaturation should increase because of the increased mVO_2. This could explain the changes in Chapter 5, in which desaturation and resaturation volume and kinetics increased.

On the other hand, because of possible perturbations of the intracellular milieu after strenuous exertion, which alters mitochondrial function (Tonkonogi et al. 1998). Muscle damage also results in increased mithochondrial Ca^{2+} concentration (Duan et al 1990). The ability of mitochondria to extract oxygen, therefore, might decrease after heavy sessions of eccentric exercise. Mitochondrial oxidative stress resulting from excess reactive oxygen species along with other factors such as temperature, catecholamine, creatine kinase etc, might also slow the oxygen desaturation kinetics during isometric contractions. In addition, the intramuscular pressure and the water content of muscle might also increase due to repetitive contractions, which could result in muscle swelling and inflammation. Although this inflammation might not be an obstacle for blood flow during rest, it could decrease oxygen diffusion from capillary to muscle fibre during isometric contractions. Generally, during isometric contractions the intramuscular pressure may increase, which in turn compresses arterioles and venules along the muscles fascicular lines. These changes, consequently, restrict arterial blood flow, increase venous out flow and decrease venous blood volume (Maguire et al. 2007).

Collectively, a decrease in oxygen availability (due to reduced diffusion) and oxygen uptake (because of mitochondrial disturbance) could result in a decreased desaturation and resaturation volume and kinetics during post-exertion isometric contractions. This assumption would tend to support the findings of Chapters 3 and 6. However, based on the outcomes of our studies, it cannot be definitively concluded which assumption might better explain the changes in oxygen saturation during isometric exercise after heavy sessions of eccentric and concentric exercise. Clearly, further research into this question is warranted.

Day-to-Day effects of exercise on muscle oxygenation

The day-to-day effect(s) of exercise (especially eccentric exercise) on muscle oxygenation and blood flow has not been thoroughly investigated. Additionally, different findings have been reported by those groups that had addressed this issue. For instance, Sbriccoli et al. (2001) and Laaksonen et al. (2006) observed a marked increase in local blood flow after EE. Kano and colleagues (2005), in an animal study, have also demonstrated that downhill running impaired muscle microcirculatory flow, as well as the balance between oxygen delivery and consumption at the onset of exercise. In contrast, Walsh et al. (2001) did not find any significant changes in oxygen utilization or local oxygen transport after eccentric cycling in humans. The dissimilarity between the findings of the previous studies suggested the multi-day assessment of muscle oxygenation during rest and exercise after a preceding bout of strenuous exercise was warranted.

Resting responses

In Chapter 3, there were significant chronic adaptations in muscle oxygenation that were persistent for several days after vigorous EE. Mean O_2Sat, which increased by 21% after acute EE, remained elevated by 5-9% for the following 6 days. As was shown by Figure 2 in Chapter 3, the increments in O_2Sat were statistically significant on days 2, 3, 5 and 7. In contrast, in Chapter 5, O_2Sat did not increase, but marginally decreased on days 4 and 5 after downhill walking (average 3.5% decrease). In Chapter 6, O_2Sat

did not change significantly within the next days after a strenuous concentric exercise session - an expected outcome. Furthermore, there were no significant long-term changes in mBF and mVO_2 in any of the experiments, although they were marginally higher than the baseline values within the days after eccentric exercise in Chapters 3 and 5 (Figure 3 in Chapter 3 and Figure 4 in Chapter 5, respectively).

Some of the factors that might affect muscle oxygenation after EE are presented in Figure 3. These include membrane permeability, blood flow, swelling, intramuscular pressure, capillary damage, and energy requiring process for healing. These factors could increase or decrease mVO_2; therefore, the final modification in muscle oxygenation could be the sum of these negative and positive effects. Although there is not a certain explanation for the observations that resting O_2Sat increased in Chapter 3, several possible mechanisms might be implicated. Firstly, muscle damage like any other kind of injury requires additional energy for the healing and recovery processes. To provide this extra energy, additional oxygen is needed, which could be provided by an increase in available O_2Sat. A higher O_2Sat could be prepared by an increase in blood flow to the damage area. Secondly, prolonged alteration of muscle oxygenation and haemoglobin-associated measures after EE, could be a local muscle effect or a reflection of some inflammatory changes induced by EE, resulting in increased blood redistribution to the affected limb. Thirdly, mitochondrial function could be impaired due to the possible perturbations of the intracellular milieu resulting from heavy exercise (Tonkonogi et al. 1998). This might reduce the ability of mitochondria to extract O_2, which consequently might increase O_2Sat. In addition, impaired capillary perfusion may lower the availability of oxygen to the muscle fibres and slow muscle oxygen consumption, which might result in an increased O_2Sat. Kano et al. (2005) reported an impaired capillary perfusion, with decreased capillary red blood cell velocity and decreased proportion of capillaries with continuous flow, up to 3 days after eccentric exercise in rodent spinotrapezius muscle. Finally, EE might increase the intramuscular pressure and the water content of the muscle (Friden et al. 1983). This could increase the diffusion distance of O_2 from capillaries to mitochondria and result in a decreased O_2 extraction and an increased O_2 availability (leading to an increased O_2Sat).

It is worthwhile to note that the above mechanisms could be applied to the eccentric protocols only, because of their potential to evoke damage. Muscle damage, as was defined in this dissertation, was observed in Chapters 3 and 5, although the magnitude of effect in Chapter 5 was lower than in Chapter 3. There were no sign of muscle damage in Chapter 6, therefore, no change was expected to occur in O_2Sat after concentric exercise, and this was supported by our findings.

However, a similar pattern of change in muscle oxygenation was expected within Chapters 3 and 5. The reason for the dissimilarity of results between these tow Chapters is unclear. However, the disparity of findings could be due to the different experimental protocols applied in these studies. In Chapter 3, the biceps brachii of participants undertook 70 maximal eccentric exercises, which evoked more muscle damage compared to the downhill exercise, as was evidenced by the changes in CK and MVT. Consequently, different levels of changes in muscle oxygenation might have occurred. Furthermore, in Chapter 3, the NIRS probe was placed over the belly of biceps brachii, which was the main muscle affected by EE. In Chapter 5, the NIRS probe was positioned on vastus lateralis, which was one of the few muscles involved in downhill walking that might be implicated in muscle damage. Previous studies have shown that there were different levels of muscle oxygenation in different agonist muscles performing similar exercises (Azuma et al. 2000; Hiroyuki et al. 2002). In addition, a particular tissue region monitored by NIRS might differ in blood flow and metabolic rate from the average value of a particular body segment (Boushel et al. 2001). Finally, muscle oxygenation could change because of the many factors affecting mitochondrial respiration (Figure 1). The outcome of the positive and negative effects of these factors might be different in downhill walking compared to eccentric elbow flexion.

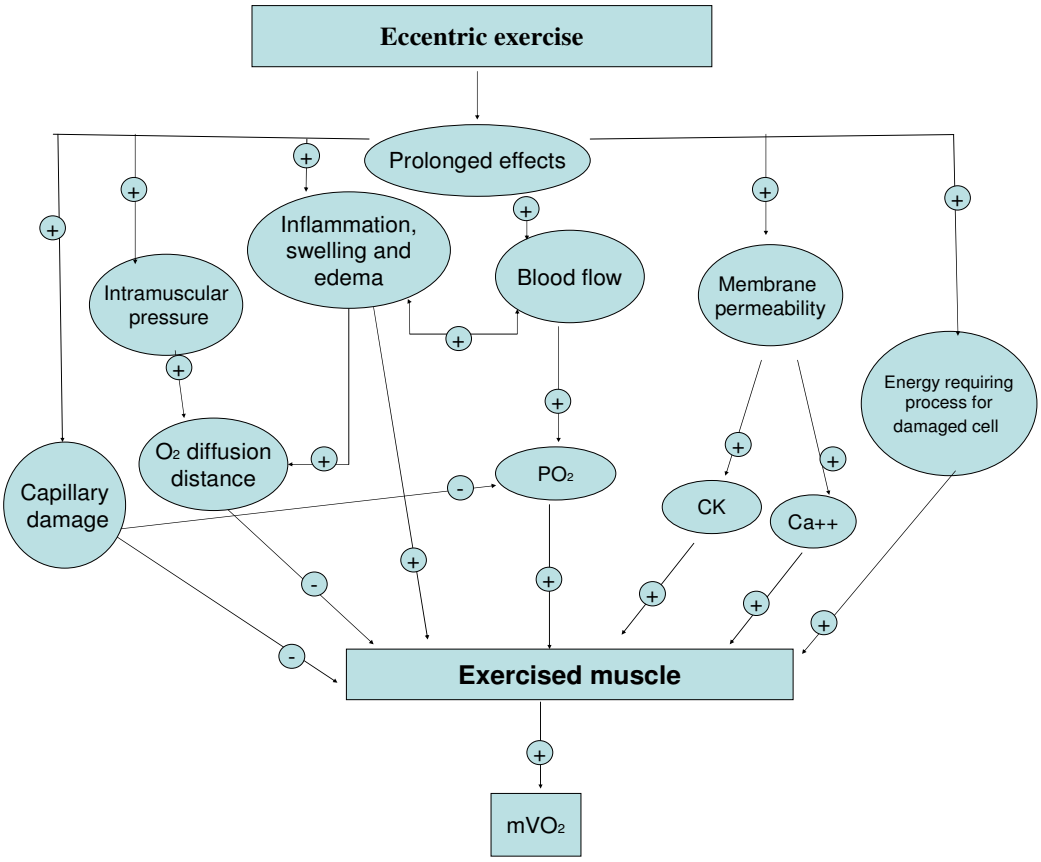

Figure 3. A proposed model for some of the many factors affecting muscle oxygen consumption (mVO_2) after eccentric exercise. O_2 refers to oxygen and PO_2 refers to oxygen pressure. Arrows refer to the direction of effect, + refers to increasing effect and – refers to decreasing effect.

During super-systolic arterial occlusions in Chapter 5, Tau-deO_2Sat increased significantly after downhill walking and remained elevated until day 4 (Table 1; Chapter 5). ΔdeO_2Sat also increased significantly after the exercise session and was significantly higher than the baseline value on days 2 and 5 (Table 1; Chapter 5). However, there was no significant change in ΔreO_2Sat and Tau-reO_2Sat during super-systolic arterial occlusions in Chapter 5 (Table 1; Chapter 5). As was expected from the non-damaging nature of concentric exercise, there were no long-term changes in O_2Sat during arterial occlusions in Chapter 6.

The short-term increments in oxygen uptake during arterial occlusion, as was described earlier, could be attributed to an increase in post-exercise oxygen consumption. However, the prolonged increases in these measures could be due to the factors that contributed to the long-term increase and decrease in O_2Sat and mVO_2, respectively (some of these factors are presented in Figure 3). The observation that the long-term changes in oxygen desaturation and resaturation during post-exercise super systolic occlusions occurred only after EE protocol, further supported our hypotheses that eccentric exercise alters the pattern of muscle oxygenation.

Exercise responses

In all studies presented in this dissertation, the changes in O_2Sat were assessed during isometric contractions of given intensities. In Chapter 3, the NIRS data obtained during both IC50 and IC80 showed significant reductions over post-EE days for all NIRS-derived variables (except for Tau-reO_2Sat). Again, these adaptations suggested a slower and a lesser amount of desaturation and resaturation within the Exercise limb for some days after EE. The decrements in oxygen saturation volume and kinetics may indirectly reflect the reductions in mVO_2. It was previously explained that after heavy sessions of eccentric exercise, the ability of mitochondria to extract oxygen from capillaries might decrease and this could be reflected by an increase in resting O_2Sat, which was observed in Chapter 3. If disturbances in mitochondria had occurred and thereby, mVO_2 had decreased, it could also be expected that during isometric contractions in eccentrically exercised muscles the mVO_2 should decrease. This was also supported by the decrements in oxygen desaturation and resaturation volume and kinetics that were observed in Chapter 3. The disturbances in muscular oxidative capacity (Newcomer et al. 2005) and microvascular (Kano et al. 2005) after EE have been previously reported. Kano and colleagues (2005) suggested that one consequence of the microcirculatory disturbance after eccentric exercise could be slowed VO_2 kinetics, which might be associated with reduced contractile function and impaired exercise tolerance.

Interestingly, according to the findings of Chapter 3, after EE the Exercise limb performed a given task (i.e. IC50 and IC80) at a lower mVO_2 compared to pre-EE. One

might speculate how a damaged muscle could be more efficient in term of its oxygen consumption. As noted in Chapter 6, one possibility was that the Exercise muscle was recruiting different muscle units and/or fibre types to those that were used in pre-EE session. For instance, after the EE session the muscle might have recruited more fast-twitch fibres compared to the pre-EE condition. Fast-twitch fibres require and consume less oxygen. However, the findings of Chapter 4 (a decrease in the EMG median frequency possibly due to a lesser recruitment of fast-twitch fibres) did not support this assumption. Another possibility was that because of the possible damage in biceps brachii, the Exercise limb had involved other flexor muscles of forearm and wrist more than the pre-exercise session. This might have reduced the contribution of biceps brachii for force production, and therefore, it might lead to a decrease in mVO_2.

As was described in Chapter 5, during isometric contractions after downhill exercise there were either no changes or some increases in oxygen desaturation and resaturation volume and kinetics (Table 1 and Figure 5; Chapter 5, respectively). These results were in contrast to those of Chapter 3 and the reason(s) for this dissimilarity is unclear. Nevertheless, some possible mechanisms that were explained earlier in this Chapter, could contribute to this divergence. The data supporting muscle damage was less severe in Chapter 5 vs. Chapter 3, and this could have affected the outcomes. The minor increases in oxygen desaturation and resaturation volume and kinetics might indicate that the mVO_2 required for isometric contractions has increased due to downhill walking exercise. In other words, after a session of downhill walking the vastus lateralis muscle consumed more oxygen to perform a given task (isometric contraction at certain intensities) within a certain time (20s) compared to pre-downhill walking. This could be because of the possible increments in energy requirements for damage-recovery process(es), and increased blood flow and cell membrane permeability (Figure 2). Collectively, the findings of Chapter 5 might reflect that after downhill walking, the vastus lateralis muscle was less efficient in term of energy efficiency. Although this finding seemed to have a better rationale compared to that of Chapter 3, it might not explain the possible perturbations in mitochondrial respiration evoked by EE, which might decrease mVO_2 (i.e. if there were mitochondrial disruptions, they would not be capable of extracting additional oxygen from capillaries). Furthermore, the changes

observed in Chapter 5 were only marginal, while in Chapter 3, significant changes were observed for several days.

Similar to its resting values, there were no prolonged changes in oxygen desaturation and resaturation kinetics and volume during IC30, IC50 or IC80 in Chapter 6. There were no prolonged changes in mechanical and biochemical characteristics of the exercised muscle in Chapter 6, therefore, no long-term alteration in muscle oxygenation were expected.

Finally, it should be noted that although isometric contractions themselves could reduce muscle oxygenation due to the increase in intramuscular pressure (De Ruiter et al. 2007), this would not affect our results. Our subjects performed similar contractions over a period of several days and their results were compared day-to-day. That means, if there were a change in muscle oxygenation during isometric contractions, it would be the same over the following days. Therefore, the differences in oxygen desaturation and resaturation observed in Chapters 3 and 5 could for the most part be due to the EE protocol on day 1.

Conclusions

Generally, after exercise, there is a demand for additional oxygen consumption. The amount and the duration of this supplementary oxygen uptake that has been called EPOC in the recent literature, depend on the exercise duration, type and tension, as well as the training state of each individual. The main purpose of this dissertation was to examine the muscle oxygenation responses to strenuous sessions of eccentric and concentric exercise. The studies presented within this thesis adhered to four primary objectives. The first objective was to assess the changes in muscle oxygenation and blood flow for six days after eccentric contractions of the arm. The second objective was to investigate the myoelectrical behaviour of biceps brachii muscle for six days after eccentric exercise. The third objective was to monitor muscle oxygenation and blood flow for four days after a session of downhill walking exercise. The fourth

objective was to examine muscle oxygenation and blood flow for two days after a session of concentric exercise.

In summary, for the first time in humans, the findings of this dissertation revealed significant and prolonged changes in muscle oxygenation during exercise or post exercise recovery, which occurred following sessions of strenuous eccentric exercise. The pattern of long-term changes in muscle oxygenation after eccentric contractions of biceps brachii muscle was not similar to that of vastus lateralis muscle after downhill walking. Although not certain, some of the possible reasons for this divergence have been discussed herein.

Some of the possible mechanisms responsible for the changes in muscle oxygenation after EE are as follows: (1) increased resting muscle oxygen utilization due to probable muscle damage and a subsequent requirement of energy demanding repair processes; (2) increased resting oxygen saturation due to the increased blood flow and/or the decreased mVO_2. The decline in mVO_2, in this case, could be due to mitochondrial disturbance, increments in cell membrane permeability and decrements in the oxygen diffusion capacity; and (3) oedema and increased intramuscular pressure that might decrease oxygen diffusion from capillaries to mitochondria.

Although concentric exercise resulted in fatigue, as evidenced by the short-term reductions in MVT, it did not affect muscle oxygenation over several days thereafter. The changes observed in NIRS-measured variables during rest after concentric exercise could be a reflection of EPOC.

Using EMG, we also observed a significant shift of MDF intercept towards lower frequencies during isometric contractions after EE in the exercised arm. The alterations in RMS values were not consistent. Therefore, it could be suggested that a prolonged reduction in MDF intercept was a concomitant sign of muscle damage, but this was not closely time associated with biochemical, anthropometric or functional markers of muscle damage. Compared to RMS, MDF was a more consistent measure to reflect

changes in EMG after EE. Overall, the EMG analyses of day-day changes of MDF or RMS were less promising than had been originally expected.

Finally, it should be noted that at the present time, there exists no complete or universally accepted explanation of the post-exercise metabolism phenomenon. However, application of NIRS that is a non-invasive and reliable technique, in conjunction with other methods of assessing muscle oxygenation, could reveal many of those underlying mechanisms.

Hypotheses outcomes

Based on the findings of our experimental studies, the principal hypotheses of this thesis were upheld or refuted as followed:

Hypothesis 1: Muscle oxygenation would be decreased after unaccustomed eccentric contractions.

*This hypothesis was **upheld**. As the findings of Chapter 3 showed, muscle oxygen consumption increased 30-min after eccentric exercise, but recovered by the next day. On the other hand, muscle oxygen saturation increased at rest, and oxygen desaturation and resaturation amount and rate decreased during isometric contractions. This might suggest that muscle oxygenation has decreased after eccentric exercise.*

Hypothesis 2: EMG activity would be decreased due to exercise-induced muscle damage.

*This hypothesis was **upheld**. The results of Chapter 4 revealed that EMG median frequency decreased. The changes in EMG root mean square, however, were not consistent over the experimental days.*

Hypothesis 3: Downhill walking exercise would affect muscle oxygenation in a similar pattern to that of eccentric contractions of biceps brachii.

*This hypothesis was **refuted**. Tissue oxygen saturation increased after biceps eccentric exercise in Chapter 3, but this did not change or marginally decreased after downhill walking in Chapter 5. Tissue oxygen desaturation and resaturation rate and amount decreased during isometric contractions in Chapter 3, but these increased after downhill exercise.*

Hypothesis 4: Concentric contractions would not induce prolonged changes in muscle oxygenation and blood flow.

*Based on the findings of Chapter 6, this hypothesis was **upheld**. Although muscle oxygenation and blood flow increased 30-min after concentric exercise, these hadreturned to the baseline values by the next day.*

Study limitations

As described in Chapter 3, some known limitations might have affected the findings of this study. Changes in the investigated limbs' adiposity during the study period might have had a confounding effect on our results. Van Beekvelt et al. (2001) reported that adipose tissue thickness has a substantial confounding influence on in vivo NIRS measurements. However, the recent findings of Maikala and Bhambhani (2006) did not support this issue. Although in Chapters 3 and 5, we did not observe a significant correlation between limbs skinfold thickness and resting O_2Sat, mVO_2 or mBF, there were some correlations between these variables in Chapter 6. In the studies presented in this manuscript, we measured skinfold adiposity only on day 1 prior to exercise and no correlation analyses could be performed on the subsequent days.

Another limitation to this study might be the effect of skin temperature on NIRS-derived variables. This issue has recently raised concerns amongst some researchers, although a definitive relationship-bias between NIRS-derived data and skin temperature has yet to be proven. Davis et al (2006) and Buono and colleagues (2005) have recently reported that the contribution of skin blood flow to NIRS measurements of muscle

oxygenation can be significant and can potentially confound interpretation of the NIRS data. In contrast, skin temperature bias has been disputed by Ferrari and colleagues (2006). In our studies, we did not monitor the changes in the skin temperature; however, all studies were performed in a similar environmental temperature.

Acute responses 'at rest' were actually 30-min recovery post exercise. This approach was chosen to ensure enough passive rest after exhaustive eccentric and concentric exercise. This might have underestimated some of the immediate effects of vigorous exercise.

Isometric contractions of different intensities may have confounded the results in both Control and Exercise limbs.

In each experiment, only one muscle was assessed for muscle oxygenation and blood flow. Movement synergists alter their responsibilities. This might have confounded the outcomes of this dissertation.

Finally, in our experiments we did baseline measurements only on day 1 before exercise. This may affect the reliability of our findings. However, reliability and validity of NIRS outcomes have been previously reported (e.g. Hampson and Piantadosi. 1988; Van Beekvelt et al. 2001; Sako et al. 2001; Kell et al. 2004; Murthy et al. 1997). Besides, we employed control arms for the duration of Chapters 3 and 5, which could be applied as substitutes for baseline measurements. In addition, in order to prevent the possible training effect on the outcomes, prior to each experiment subjects were habituated to the equipment and procedures used in the experiment.

Future research directions

Very few studies have attempted to investigate the effect of eccentric exercise on oxidative metabolism and muscle oxygenation. The findings of this study showed some prolonged changes in muscle oxygenation after EE. However, there were some dissimilarities between the outcomes of the two eccentric protocols we applied in our

studies. This might raise new questions about the true effect of EE on muscle oxygenation. Therefore, further investigation is warranted to identify the underlying mechanisms supporting changes in muscle oxygenation kinetics after EE, and to elucidate whether these are affected by or affecting EE. We suggest a future study with a similar protocol to that of Chapter 3 using NIRS in conjunction with other techniques such as magnetic resonance imaging, phosphorescence quenching, plethysmography etc.

Based on the findings of our studies, it is also suggested that future research investigate the changes in muscle oxygenation not only in the main muscles (such as biceps brachii in Chapter 3) but also in other agonist and antagonist muscles involved in EE, using regional NIRS mapping. Finally, avoiding the limitations of the studies presented in this thesis, could further improve the quality of a future research.

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Appendices

Appendix 1

Authors instructions for Applied Physiology, Nutrition and Metabolism

The manuscript

Format and organization

The manuscript should be typewritten, double-spaced, on paper 8.5 × 11 in. (or ISO A4). Typing should be on one side of the page only. Each page should be numbered, beginning with the title page. For material that is to be set in italics, use an italic font; do not underline. Use capital letters only when the letters or words should appear in capitals.

All manuscripts (other than Book Reviews and Abstracts) should contain a title page (p. 1), an abstract (p. 2), followed by Introduction (p. 3), Materials and methods, Results, Discussion, and Acknowledgements sections, plus references, tables, figure captions, and appendices, in that order. (See descriptions of each part of the manuscript, below.) Tables and captions for illustrations should be on separate pages. Primary headings indicate the major sections of the paper (Introduction, Materials and methods, Results, Discussion, Acknowledgements, References). Secondary headings indicate major divisions within a primary section. Tertiary headings indicate divisions within a secondary heading.

Organize tables and figures to facilitate comparisons, grouping related data in as few tables and figures as feasible. As far as possible, make the tables and figures clear without reference to the text.

Begin sections and paragraphs with topic sentences containing generalizations that lead readily to the particulars. Giving a conclusion first and then supporting it not only improves readability but also facilitates assessment by other scientists. Failure to give the most newsworthy generalizations first is one of the most prominent shortcomings in

presentation of manuscripts. Assure that everything in each section is relevant to the heading and that everything in each paragraph is relevant to the topic (opening) sentence.

Title

Both titles and abstracts provide information for contemporary alerting and information retrieval services, and should therefore be informative but brief.

Title page

The title page should contain the following: *(i)* The full title of the paper. *(ii)* Authors listed in the order in which they are to appear at the head of the printed article. *(iii)* Affiliation and address (including e-mail address) for each author. This should reflect the affiliation and address at the time of the study. Indicate current affiliations and addresses (including e-mail addresses) that differ from those in the by-line in a footnote. *(iv)* Name, address, telephone number, fax number, and e-mail address of the author responsible for correspondence.

Author names

The Editors urge all authors to use full forenames rather than initials and (or) one forename.

Abstract

An abstract is required for every contribution and should contain accurate descriptive words that will draw the reader to the content. This is particularly important because contemporary alerting services and search engines will search this text. The concise abstract should present the paper content accurately and should supplement, not duplicate, the title in this respect. Authors able to submit abstracts in both fluent English and French are encouraged to do so. Abstracts submitted in one language will be translated into the other official language by the journal translator. References should not be cited in the abstract unless they are absolutely essential, in which case full bibliographic information must be provided. **Original research** communications must

have a **structured abstract** of no longer than 250 words with the following sections: (*i*) Background, (*ii*) Objective, (*iii*) Research design and methods, (*iv*) Results, and (*v*) Conclusions, whereas **reviews and invited reviews** should have an **unstructured abstract** of 200 words or less. Rapid communications, brief communications, current opinions, and technical notes should have an unstructured abstract of no more than 75 words, whereas invited editorials and letters to the editor do not have abstracts.

Key words

Six to 10 key words should be placed directly below the abstract.

Text

The text should be written and arranged to ensure that the observations reported may be reproduced and (or) evaluated by readers. Sources of biological materials, experimental methods, geographical locations, and statistical methods should be described. Precise locations of rare and endangered organisms should not be divulged. Sources of commercially available laboratory or field equipment and fine chemicals should be indicated in parentheses; list the company name, city, and country. Material taken from research theses must be thoroughly edited for brevity and must conform to these Instructions to Authors. Authors are encouraged to include uniform resource locators (URLs) and digital object identifiers (DOIs) to enable readers to find material on the World Wide Web. URLs and DOIs for references cited should be placed after the reference in the reference list; other URLs should be placed in context in the text.

Introduction

Limit the introduction largely to the scope, purpose, and rationale of the study. Restrict the literature review and other background information to that needed in defining the problem or setting the work in perspective. Try beginning with the purpose or scope of the work, defining the problem next, and adding guideposts to orient the reader. An introduction generally need not exceed 375–500 words.

Materials and methods

The degree of reproducibility of experiments should be indicated either in general statements in Materials and methods and Results or, preferably, as statistical treatments of numerical data cited in tabular or graphic form. The experimental, or computational, material must be sufficiently detailed to permit reproduction of the work, but must be concise and avoid lengthy descriptions of known procedures; the latter should be specified by appropriate references. A reader's attention should be drawn to any new or unusual hazards encountered in the experimental work. Materials and methods provides the framework for getting answers to the questions posed in the purpose of the work. Limit the information on materials and methods to what is needed in judging whether the findings are valid. To facilitate assessment, give all the information in one section when possible. Refer to the literature concerning descriptions of equipment or techniques already published, detailing only adaptations. If the section is long, consider using subheadings corresponding to headings for the findings. Identify figures that have been digitally enhanced or modified, and provide the software and technique used.

Results

Limit the results to answers to the questions posed in the purpose of the work and condense them as comprehensively as possible. Give the findings as nearly as possible in the terms in which the observations or measurements were made so as to avoid confusion between facts and inferences. State noteworthy findings to be noted in each table and figure, and avoid restating in the text what is clear from the captions. Material supplementary to the text can be archived in the report literature or a recognized data depository and referenced in the text (see Supplementary material section).

Discussion or conclusion

Limit the Discussion to giving the main contributions of the study and interpreting particular findings, comparing them with those of other workers. Emphasis should be maintained on synthesis and interpretation and exposition of broadly applicable generalizations and principles. If these are exceptions or unsettled points, note them and show how the findings agree or contrast with previously published work. Limit

speculation to what can be supported with reasonable evidence. End the Discussion with a short summary of the significance of the work and conclusions drawn.

Acknowledgements

Acknowledgements should be written in the third person and kept to a concise recognition of relevant contributions. We strongly urge authors to limit acknowledgments to those who contributed substantially to scientific and technical aspects of the paper, gave financial support, or improved the quality of the presentation. Avoid acknowledging those whose contribution was clerical only.

Footnotes

Footnotes to material in the text should not be used unless they are unavoidable, but their use is encouraged in tables. Where used in the text, footnotes should be cited in the manuscript by superscript Arabic numbers (except in the tables, see below) and should be numbered serially beginning with any that appear on the title page. Each footnote should be typed on the manuscript page upon which the reference is made; footnotes should not be included in the list of references.

Equations

Equations should be clearly typed; triple-spacing should be used if superscripts and (or) subscripts are involved. Superscripts and subscripts should be legible and carefully placed. Distinguish between lowercase l and the numeral one, and between capital O and the numeral zero. A letter or symbol should represent only one entity and be used consistently throughout the paper. Each variable must be defined in the text. Numbers identifying equations must be in square brackets and placed flush with the left margin.

References

General form

The author is responsible for verifying each reference against the original article. Each reference must be cited in the text using the surnames of the authors and the year, for

example, (Walpole 1985) or (Green and Brown 1990) or Green and Brown (1990). Depending on the sentence construction, the names may or may not be in parentheses, but the year always is. If there are three or more authors, the citation should give the name of the first author followed by et al. (e.g., Green et al. 1991). If references occur that are not uniquely identified by the authors' names and year, use *a*, *b*, *c*, etc., after the year, for example, Green 1983*a*, 1983*b*; Green and Brown 1988*a*, 1988*b*, for the text citation and in the reference list.

Uniform resource locators (URLs) or digital object identifiers (DOIs) are useful in locating references on the Web, and authors are encouraged to include these; they should be added to the reference in the reference list (see example below).

Unpublished reports, private communications, and In press references

References to unpublished reports, private communications, and papers submitted but not yet accepted are not included in the reference list but instead must be included as footnotes or in parentheses in the text, giving all authors' names with initials; for a private communication, year of communication should also be given (e.g., J.S. Jones (personal communication, 1999)). If an unpublished book or article has been **accepted for publication**, include it in the reference list followed by the notation "In press".

Presentation of the list

The reference list must be double-spaced and placed at the end of the text. References must be listed in alphabetical order according to the name of the first author and not numbered. References with the same first author are listed in the following order. (*i*) Papers with one author only are listed first in chronological order, beginning with the earliest paper. (*ii*) Papers with dual authorship follow and are listed in alphabetical order by the last name of the second author. (*iii*) Papers with three or more authors appear after the dual-authored papers and are arranged chronologically.

General guidelines on references

References should follow the form used in current issues of the Journal. The names of serials are abbreviated in the form given in the *List of Journals Indexed for MEDLINE*

(National Library of Medicine, National Institutes of Health, 8600 Rockville Pike, Bethesda, MD 20894, USA; http://www.nlm.nih.gov/tsd/serials/lji.html). In doubtful cases, authors should write the name of the serial in full. The Journal encourages the inclusion of issue numbers, which should be placed in parentheses after the volume number. References to nonrefereed documents (e.g., environmental impact statements, contract reports) must include the address where they can be obtained. The following bibliographic citations illustrate the punctuation, style, and abbreviations for references.

Journal article

Redwood, R.G., and Jain, A.K. 1992. Code provisions for seismic design for concentrically braced steel frames. Can. J. Civ. Eng. **19**(9): 1025–1031.

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van der Sanden, J.J., and Hoekman. D.H. 2005. Review of relationships between grey-tone co-occurrence, semivariance, and autocorrelation based image texture analysis approaches [online]. Can. J. Remote Sensing, 31(3): 207–213. Available from http://pubs.nrc-cnrc.gc.ca/cjrs/rs3-05.html [accessed 9 September 2005].

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Gordon, D.C., Jr., and Hourston, A.S. (*Editors*). 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish. Aquat. Sci. 40(Suppl. 1).

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Sanders, W.W., Jr., and Elleby, H.A. 1970. Distribution of wheel loads in highway bridges. National Cooperative Highway Research Program Report 83, Transportation Research Board, National Research Council, Washington, D.C.

Book

Williams, R.A. 1987. Communication systems analysis and design. Prentice-Hall, Inc., Englewood Cliffs, N.J.

Book in a series

Scott, W.B., and Crossman, E.J. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. No. 184.

Part of book

Healey, M.C. 1980. The ecology of juvenile salmon in Georgia Strait, Britsh Columbia. *In* Salmonid ecosystems of the North Pacific. *Edited by* W.J. McNeil and D.C. Himsworth. Oregon State University Press, Corvallis, Oreg. pp. 203–229.

Paper in conference proceedings

Kline, V.M., and McClintock, T. 1994. Effect of burning on a dry oak forest infested with woody exotics. *In* Proceedings of the 13th North American Prairie Conference: Spirit of the Land, Our Prairie Legacy, Windsor, Ont., 6–9 August 1992. *Edited by* R.G. Wickett, P.D. Lewis, A. Woodcliffe, and P. Pratt. Department of Parks and Recreation, Windsor, Ont. pp. 207–213.

Institutional publications and pamphlets

Dzikowski, P.A., Kirby, G., Read, G., and Richards, W.G. 1984. The climate for agriculture in Atlantic Canada. Available from the Atlantic Advisory Committee on Agrometeorology, Halifax, N.S. Publ. ACA 84-2-500. Agdex No. 070.

Corporate author

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1975. Standard methods for the examination of water and wastewater. 14th ed. American Public Health Association, American Water Works Association, and Water Pollution Control Federation, Washington, D.C.

Thesis

Keller, C.P. 1987. The role of polysaccharidases in acid wall loosening of epidermal tissue from young *Phaseolus vulgaris* L. hypocotyls. M.Sc. thesis, Department of Botany, The University of British Columbia, Vancouver, B.C.

Electronic citation

Quinion, M.B. 1998. Citing online sources: advice on online citation formats [online]. Available from http://www.worldwidewords.org/articles/citation.htm [accessed 20 October 2005].

Translation

Koike, A., and Ogura, B. 1977. Selectivity of meshes and entrances of shrimp traps and crab traps. J. Tokyo Univ. Fish. 64: 1–11. [Translated from Japanese by Can. Transl. Fish. Aquat. Sci. 4950, 1983.]

Tables

Tables must be typed on separate pages, placed after the list of references, and numbered with Arabic numerals in the order cited in the text. The title of the table should be a concise description of the content, no longer than one sentence, that allows the table to be understood without detailed reference to the text. Column headings should be brief, but may be amplified by footnotes. Vertical rules should not be used. A copy of the Journal should be consulted to see how tables are set up and where the lines in them are placed. Footnotes in tables should be designated by symbols (in the order *, †, ‡, §, ‖, ¶ , #) or superscript lowercase italic letters. Descriptive material not

designated by a footnote may be placed under a table as a Note. Numerous small tables should be avoided, and the number of tables should be kept to a minimum.

Figure captions

Figure captions should be listed on a separate page and placed after the tables. The caption should informatively describe the content of the figure, without need for detailed reference to the text. Experimental conditions should not be included, but should be adequately covered in the Methods. For graphs, captions should not repeat axis labels, but should describe what the data show. A single caption can be provided for multipart (composite) figures, with necessary details on the separate parts, identified by their individual labels. If the separate parts require enough information to warrant separate captions, then the composite should be separated into individual figures.

Appendices

An appendix should be able to stand alone, as a separate, self-contained document. Figures and tables used in an appendix should be numbered sequentially but separately from those used in the main body of the paper, for example, Fig. A1, Table A1, etc. If references are cited in an appendix, they must be listed in an appendix reference list, separate from the reference list for the article.

Supplementary material

Supplementary material (or data) consists of extra tables, figures (maps), detailed calculations, and data sets produced by the authors as part of their research, but not essential for understanding or evaluating the paper, and not published with the article in the print edition of the journal. Depending on the policy of the journal, such material may or may not be peer reviewed with the article. Supplementary material should be submitted with the article. During Web submission (OSPREY), relevant files should be attached under “Supplementary data”. For mail submission, supplementary material should be clearly marked as such. The National Research Council of Canada maintains a depository in which supplementary material may be placed, either at the request of the author or at the suggestion of the Editor. In addition, supplementary material can now

be made available in its native file format on the journal Web site. It will be linked from the Web page of the associated article. Tables and figures should be numbered in sequence separate from those published with the paper (e.g., Fig. S1, Table S1). The supplementary material should be referred to in the printed article by footnotes. Copies of material in the depository may be purchased from the Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, ON K1A 0R6, Canada.

Computer programs

It is not the policy of the Journal to publish detailed printouts of computer program statements. Where the availability of these details enhances the usefulness of the paper, the author should submit two copies of the program for deposit (see Supplementary material section).

Illustrations

General

Original figures should not be submitted at this time, but will be requested when the manuscript is returned for revision. However, all copies must be of similar quality to the originals, otherwise the review process may be compromised. When submitting photographs, please submit four **copies of originals**. Manuscripts with substandard photographs or photocopies will be returned or held until suitably high-quality images are provided.

Each figure or group of figures should be planned to fit, after appropriate reduction, into the area of either one or two columns of text. The maximum finished size of a one-column illustration is 8.6 × 23.7 cm (3.4 × 9.3 in.) and that of a two-column illustration is 18.2 × 23.7 cm (7.2 × 9.3 in.). The figures (including halftones) must be numbered consecutively in Arabic numerals, and each one must be referred to in the text and must be self-explanatory. All terms, abbreviations, and symbols must correspond with those in the text. Only essential labelling should be used, with detailed information given in the caption. For **hard-copy versions**, each illustration must be identified by the figure

number and the authors' names on the back of the page or in the left-hand corner, well away from the illustration area.

Line drawings

All lines must be sufficiently thick (0.5 points minimum) to reproduce well, and all symbols, superscripts, subscripts, and decimal points must be in good proportion to the rest of the drawing and large enough to allow for any necessary reduction without loss of detail. Avoid small open symbols; these tend to fill in upon reproduction. Lettering produced by dot matrix printers or typewriters, or by hand, is not acceptable. The same font style and lettering sizes should be used for all figures of similar size in any one paper. Original recorder tracings of NMR, IR, ESR spectra, etc., are not acceptable for reproduction; they must be redrawn. For **hard-copy versions**, line drawings should be made with black ink or computergenerated in black on high-quality white paper or other comparable material; laser prints should be created at the highest resolution available.

Photographs

Photographs should be continuous tone, of high quality, and with strong contrast. Only essential features should be shown. A photograph, or group of them, should be planned to fit into the area of either one or two columns of text with no further reduction. Electron micrographs or photomicrographs should include a scale bar directly on the print. The best results will be obtained if the authors match the contrast and density of all figures arranged as a single plate. **Hard-copy versions** must be printed on glossy paper and be trimmed and mounted on thin flexible white bristol board with no space between those arranged in groups.

Colour illustrations

Colour illustrations will be at the author's expense. Further details on prices are available from Judy Busnarda, Managing Editor of the Journal (e-mail: judy.busnarda@nrc-cnrc.gc.ca).

Preparation of electronic graphic files

General

NRC Research Press prefers the submission of electronic illustration files for accepted manuscripts and will use these electronic files whenever possible.

If electronic files are not available or if those supplied are inadequate for reproduction, hard-copy originals of adequate quality, either previously supplied or requested from the author, will be scanned. Note that the scanner will easily reproduce flaws (e.g., correction fluid, smudges). Submission of noncontinuous (screened) photographs and scanned illustrations printed out on laser printers is not recommended, as moirés develop; a moiré is a noticeable, unwanted pattern generated by rescanning or rescreening an illustration that already contains a dot pattern.

If sending hard copies, please ensure that electronic files match the hard copies (i.e., figure number and figure content). If sending a disk, on the disk label, identify (*i*) the software application and version and (*ii*) file name(s), size, and extension. If you have compressed your files, indicate what compression format was used. PC or Macintosh versions of True Type or Type 1 fonts should be used. Do not use bitmap or nonstandard fonts. Electronic graphics can be accepted on the following disks: 3½" disks, 100 MB Zip cartridge, and CD-ROM.

The preferred graphic application of NRC Research Press is CorelDraw! For other applications that can be used, see the electronic graphics list at http://pubs.nrc-cnrc.gc.ca/cgi-bin/rp/rp2_prog_e?apnm_graphics_e.html.

All figures should be submitted at the desired published size. For figures with several parts (e.g., *a*, *b*, *c*, *d*, etc.) created using the same software application, assemble them into one file rather than sending several files.

Remember that the more complex your artwork becomes, the greater the possibility for problems at output time. Avoid complicated textures and shadings, especially in vector illustration programs; this increases the chance for a poor-quality final product.

Bitmap

Bitmaps are image files produced using a grid format in which each square (or pixel) is set to one level of black, colour, or grey. A bitmap (rasterized) file is broken down into the number of pixels or picture elements per inch (ppi). Pixels per inch is sometimes referred to as dots per inch (dpi). The higher the resolution of an image, the larger the number of pixels contained within the rectangular grid.

The proper resolution should be used when submitting bitmap artwork. The minimum requirements for resolution are 600 dpi for line art, 1200 dpi for finelines (line art with fine lines or shading), 300 dpi for halftones and colour, and 600 dpi for combinations (halftones with lettering outside the photo area).

Colour

All colour files submitted must be as CMYK (cyan, magenta, yellow, and black). These colours are used in full-colour commercial printing. RGB graphics (red, green, and blue; colours specifically used to produce an image on a monitor) will not print correctly.

Vector

Vector files are image files produced using elements such as lines and shapes. Typically these files are used for line drawings.

Bitmap in vector

Bitmaps can be imported into vector/draw applications only for the purpose of adding and overlaying information, lines, text, etc. Bitmaps should not be resized, cropped, rotated, or otherwise manipulated after importing.

Multimedia

Audio and video clips in the major multimedia formats are now accepted for NRC Research Press journals published in full-text HTML. For accepted formats, see the electronic graphic list at http://pubs.nrc-cnrc.gc.ca/cgi-bin/rp/rp2_prog_e?apnm_graphics_e.html.

Manuscript guidelines

Style guides

As a general guide for biological terms, *Scientific Style and Format: The CSE Manual for Authors, Editors, and Publishers* (7th ed., 2006) published by the Council of Science Editors, 12100 Sunset Hills Rd., Suite 130, Reston, VA 20190, USA, is recommended.

Spelling

Spelling should follow *Webster's Third New International Dictionary* or the *Oxford English Dictionary*. Authors are responsible for consistency in spelling.

Abbreviations and acronyms

Acronyms should be defined when they are first mentioned in the text. Abbreviations and acronyms that are standard in the discipline need not be defined. Abbreviate terms denoting units of mass and measurement in the text only when they are preceded by numerals.

Units of measurement

SI units (Système international d'unités) should be used or SI equivalents should be given. This system is explained and other useful information is given in the *Metric Practice Guide* (2000) published by CSA International (178 Rexdale Blvd., Toronto, ON M9W 1R3, Canada). For practical reasons, some exceptions to SI units are allowed. Units such as kilocalorie, reciprocal centimetre (wave number), and atmosphere may be used for the foreseeable future.

Statistical analyses

The assumptions and (or) the model underlying any statistical analysis should be clearly stated. Symbols such as * and **, denoting levels of significance, should *not* be used except in conjunction with the actual values of the associated test statistic; actual *p* values are preferred.

Nomenclature

Enzymes

For enzyme nomenclature, *Enzyme Nomenclature (1992): Recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology* (Academic Press, San Diego, Calif.) should be followed.

Chemical

The following references are pertinent in nomenclature: *Nomenclature of Organic Chemistry, Sections A, B, C, D, E, F, and H*, Pergamon Press, London, 1979; *Nomenclature of Inorganic Chemistry*, Butterworth, London, 1971; *Quantities, Units and Symbols in Physical Chemistry*, Blackwell, London, 1987. Tentative recommendations exist for organometallic nomenclature, *IUPAC Information Bulletin* No. 31, 1973; for stereochemical designations, J. Org. Chem. 35, 2849, 1970; and for steroids, J. Org. Chem. 34, 1517, 1969. Although tentative IUPAC rules have been published for carbohydrate nomenclature (*Biochemistry*, 10, 3983, 1971), the Editors recommend the use of the British–American nomenclature (J. Org. Chem. 28, 281, 1963), until the IUPAC rules become definitive. For nomenclature not covered by international convention, the usage of the American Chemical Society should be followed, for example, The Naming and Indexing of Chemical Compounds (*Introduction to Chemical Abstracts* Subject Index 56, IN, 1962). Rigid adherence to nomenclature rules is not expected each time a compound is mentioned in a manuscript, but the approved names should be given at least once, preferably in an early part of the manuscript.

Drug trade names

Trade names of drugs may be mentioned in parentheses in the first text reference to a drug, but generic names should be used in the text, tables, and figures. When a trade name is used, it should be capitalized; generic or chemical names are not capitalized. The chemical nature of new drugs must be given when it is known. The form of drug used in calculations of doses (e.g., base or salt) should be indicated. When several drugs

are used, it may save space to include a separate paragraph in Methods or a separate table listing relevant information about all drugs employed.

Writing numbers

In long numbers the digits should be separated into groups of three, counted from the decimal marker to the left and right. The separator should be a space and not a comma, period, or any other mark, for example, 25 562 987 and not 25,562,987. In English text, the decimal marker should be a point, for example, 0.1 mL and not 0,1 mL. The decimal point in all numbers between 1 and –1, except 0, must be preceded by a 0. The sign × should be used to indicate multiplication, e.g., 3×10^6 and not $3 \cdot 10^6$.

Dates

Dates should be written in the sequence day–month–year without internal punctuation (On 9 October 1983 the…).

Appendix 2

Authors instructions for Journal of Sports Science and Medicine (JSSM)

Research and review articles, case and brief reports, letter to editors should be submitted to JSSM in the field of sports injuries, exercise physiology, sports rehabilitation, diseases and exercise, sports traumatology, sports psychology, nutrition, sports biomechanics and kinesiology, sports education. The articles are to be submitted electronically to Editor-in-Chief.

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INTRODUCTION

METHODS

RESULTS

DISCUSSION

CONCLUSION

ACKNOWLEDGMENTS: Provide information sufficient to identify sources of support, technical assistance, and intellectual contributions not associated with authorship.

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2000).

Journal article:

Akova, B., Sürmen-Gür, E., Gür, H., Dirican, M., Sarandöl, E. and Kücükoglu, S. (2001) Exercise-induced oxidative stress and muscular performance in healthy women: role of vitamin E supplementation and endogenous estradiol. *European Journal of Applied Physiology* 84, 141-147.

Journal article, article not in English:

Seker-Aygül, Z., Akova, B. and Gür, H. (2001) The relationship of stress and stress management factors with injury in soccer players. *Turkish Journal of Sports Medicine* 36, 71-80. (In Turkish: English abstract).

Journal article in press:

Gür, H., Cakin, N., Akova, B., Okay, E. and Kücükoglu, S (2002) Concentric versus combined concentric- eccentric isokinetic training: Effects on functional capacity and symptoms in patients with osteoarthrosis of the knee. *Archives of Physical Medicine and Rehabilitation,* in press.

Journal article in electronic format:

Weigand, D.A., Carr, S., Petherick, C. and Taylor, A. (2001) Motivational climate in Sport and Physical Education: The role of significant others. *European Journal of Sports Science (serial online)* 1(4), (13 screens/inclusive page), October. Available from URL: http://www.humankinetics.com/ejss

Abstract:

Gur, H., Şekir, U., Akova, B. and Kucukoglu, S. (2003) A multi-station proprioceptive exercise program in patients with bilateral knee osteoarthrosis: functional capacity, pain and sensoriomotor function. *8th Anual Congress European College of Sports Science, July 9-12, Salzburg- Austria.* Book of Abstract. 404.

Book:

Guyton, A.C. and Hall, J.E. (1996) *Textbook of medical physiology.* 9 th edition. W. B. Saunders Company, London.

Chapter in edited book:

Wilson, C.H. (1984) Exercise for arthritis. In: *Therapeutic exercise.* Ed: Basmajian, J.V. 4 th edition. Baltimor: Williams and Wilkins. 529-545.

Thesis:

Özyener, F. (2000) *Effects of work intensity on dynamics of pulmonary gas exchange during exercise in humans.* Doctoral thesis, University of London,
London. 79.

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MAIN TEXT

CONCLUSION

ACKNOWLEDGMENT

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2000). See the reference section of research article for detail.

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INTRODUCTION

CASE REPORT: A brief case report including history, physical examination and laboratory findings followed by treatment and outcome.

DISCUSION

CONCLUSION

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Appendix 3

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Preparation of papers

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Manuscripts should be written in English. Spelling should conform to *The Concise Oxford Dictionary of Current English*, 6th edn.

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Results giving concisely the observations made.

Discussion presenting succinctly the authors' interpretations of their findings in relation to previous work.

Acknowledgements of grants and assistance.

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Fischman DA. Development of striated muscle. In: *The Structure and Function of Muscle* (ed. Bourne, G. H.) (1972); vol. 1, part 1, pp. 75-148. Academic Press, New York.

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Appendix 4

Authors instructions for Isokinetics and Exercise Science

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PREPARATION OF MANUSCRIPTS

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- Body of text (divided by subheadings)
- Acknowledgements
- References

- Tables
- Figure captions
- Figures

Headings and subheadings should be numbered and typed on a separate line, without indentation.
Numbers should appear with decimal point, not comma: 12.3 and not 12,3
The units for isokinetic measurement is Nm. If the system's output is otherwise, authors are urged to convert e.g. from ft•lb into Nm.
On the other hand, there is **no** need to number the headings.
SI units should be used, i.e., the units based on the metre, kilogramme, second, etc.

Title page

The title page should provide the following information:
- Title (should be clear, descriptive and not too long)
- Name(s) of author(s); please indicate who is the corresponding author
- Full affiliation(s)
- Present address of author(s), if different from affiliation
- Complete address of corresponding author, including tel. no., fax no. and e-mail address
- Abstract; should be structured, clear, descriptive, self-explanatory and not longer than 200 words, it should also be suitable for publication in abstracting services
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Number as Table 1, Table 2 etc, and refer to all of them in the text.
Each table should be provided on a separate page of the manuscript. Tables should not be included in the text.
 Each table should have a brief and self-explanatory title.
Column headings should be brief, but sufficiently explanatory. Standard abbreviations of units of measurement should be added between parentheses.
Vertical lines should not be used to separate columns. Leave some extra space between

the columns instead.

Any explanations essential to the understanding of the table should be given in footnotes at the bottom of the table.

Figures

 Number figures as Fig. 1, Fig 2, etc and refer to all of them in the text.

Each figure should be provided on a separate sheet. Figures should not be included in the text.

Colour figures can be included, provided the cost of their reproduction is paid for by the author.

For the file formats of the figures please take the following into account:

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- do not use figures taken from the Internet, the resolution will be too low for printing
- do not use colour in your figures if they are to be printed in black & white, as this will reduce the print quality (note that in software often the default is colour, you should change the settings)
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Figures should be designed with the format of the page of the journal in mind. They should be of such a size as to allow a reduction of 50%.

On maps and other figures where a scale is needed, use bar scales rather than numerical ones, i.e., do not use scales of the type 1:10,000. This avoids problems if the figures need to be reduced.

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Photographs are only acceptable if they have good contrast and intensity.

References

For citations in the text numbers between square brackets should be used. All

publications cited in the text should be presented in a list of references following the text of the manuscript.

References should be listed alphabetically in the following style:

[1] T.D. Cahalan, M.E. Johnson and E.Y. Chao, Shoulder strength analysis using the Cybex II isokinetic dynamo, *Clinical Orthopaedics and Related Research* **271** (1991), 249-257.

[2] H.D. Hartsell, The effects of body position and stabilization on isokinetic torque ratios for the shoulder rotators, *I E S* **7** (1998/1999), 161-170.

[3] S. Lippitt, Mechanism of glenohumeral joint stability, *Clinical Orthopaedics and Related Research* **291** (1993), 20-27.

Footnotes

Footnotes should only be used if absolutely essential. In most cases it is possible to incorporate the information in the text.

 If used, they should be numbered in the text, indicated by superscript numbers and kept as short as possible.

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Appendix 5

Participant information and consent forms

The University of Sydney
Faculty of Health Sciences

Participant Information Sheet

Muscle oxygenation and muscle blood flow during and after eccentric exercise using near infrared spectroscopy

You are invited to take part in a research project titled *"Muscle oxygenation and muscle blood flow during and after eccentric exercise using near infrared spectroscopy"* being conducted by Associate Professor Glen Davis, Sirous Ahmadi and Mrs Nasim Foroughi at the Rehabilitation Research Centre within the Faculty of Health Sciences. The project will investigate how we can estimate the quantity and the quality of muscle soreness and/or damage via monitoring muscle blood flow and oxygenation. This research is conducted as a component of the PhD studies of Mr Sirous Ahmadi.

You have been selected for this study because you are male, aged 18-45, not a competitive sportsperson but otherwise healthy, and without any recent arm muscle injuries. You also must not be on any prescription medications. You will be excluded from participation if you have had an arm muscle injury (of any type) or orthopaedic injuries within the past 12 months, if you perform upper-body strength training more often than once per week, if you have had recent arm muscle biopsy, or if you cannot attend the research laboratory for eight (8) consecutive days

Background
During many sport activities and unaccustomed eccentric exercise, the muscles undertake excessive pressures, which may result in *delayed onset of muscle soreness*. The quantity and quality of exercise-induced delayed onset of muscle soreness has been evaluated both directly and indirectly. However, due to the invasive nature of direct studies, indirect methods to evaluate delayed onset of muscle soreness such as measuring changes in blood biochemistry, a person's perceived muscle soreness, their maximum voluntary strength, muscle electromyography, and other physiological measurements have been preferred to use in human studies. However, the accuracy and reproducibility of these measurements is still unclear.

Oxygen plays a crucial role in energy metabolism and the oxygenation rate changes due to metabolic problems after soreness. The structural changes, increased intramuscular pressure, or increased water content observed in muscle after eccentric exercise may restrict local blood flow and increase the diffusion distance of oxygen. Near infrared spectroscopy is a simple and non-invasive method to monitor oxygen delivery and phosphocreatine recovery in skeletal muscle in healthy and diseased humans.

What do I need to do?
In the current study, we will non-invasively measure your muscle blood flow and oxygenation before, during and after two sessions of eccentric exercise by means of

near infrared spectroscopy. Your maximum strength, some blood biochemical measures, your elbow joint range of motion, arm circumference, perceived soreness, and muscle electrical activity (electromyography) will be measured on the first day when you do strenuous arm exercise and for the following 7 days in recovery.

We will ask you to attend laboratory testing sessions for 1 hour each day over a period of 8 days. On the first day, you will perform 2 bouts of 35 maximal voluntary eccentric contractions with the elbow flexors of your non-dominant arm placed in an isokinetic dynamometer moving at a slow speed (60 deg•s^{-1}). Eccentric exercise will consist of three sets of 10 repetitions. You will be verbally encouraged to maximally resist actions in which the arm will be forcibly extended from an elbow-flexed (50°) to an elbow-extended (180°) position. The researcher will bring your arm up to the elbow-flexed position after each eccentric movement. You will perform another session of eccentric exercise similar to the first session at the seventh day.

A range of measurements will be assessed before and after eccentric exercise on day 1, and every 24hr for seven consecutive days thereafter. Blood measurements of creatine kinase will be taken via withdrawing approximately 10ml blood from a vein in your arm by a researcher skilled with blood drawing techniques before and after the first eccentric exercise session, and at days 3, 5 and 7 thereafter. This is a simple and minimally invasive procedure that will be done via stringent aseptic methods by experienced personnel to prevent complications. Blood creatine kinase is collected because it is a biochemical marker of muscle soreness. Furthermore, near infrared spectroscopy and electromyography measurements (described below) will be done during the eccentric exercise session.

Your relaxed and flexed elbow joint angles will be assessed three times using an angle-measuring device (called a “goniometer”), and the difference between the two angles will be used as your joint range of motion around the elbow joint. We will measure your arm circumference at 4, 6, 8, and 10 cm above the elbow joint, while allowing your arm to hang down by the side. Soreness when palpating the upper arm during flexion and extension of the elbow joint, as sometimes occurs with delayed onset of muscle soreness, will be evaluated by a visual analog scale that has a 100-mm continuous line with "not sore at all" on one side (0 mm) and "very, very sore" on the other side (100 mm). Finally, your maximum voluntary contraction will be measured with your elbow joint at 90° whereby 3-second repetitions (3 times) will be performed with 1 min of rest between each contraction. We also will evaluate your muscle oxygenation and Electromyographic signals while you are performing 3 bouts of isometric contractions, each contraction lasts 20 seconds with 80% of maximum voluntary contraction.

Surface electrical signals from your muscles electromyography signals will be recorded from the biceps brachii muscle of your non-dominant arm. The skin will be prepared by

shaving, abrading, and cleaning with alcohol. Small electrodes will be fastened over the belly of the biceps muscle and we will place a reference electrode on the left side of your shoulder. The electrode positions will be marked with a semi-permanent marker to assure standardized measurements from day to day.

Dual-channel near infrared spectroscopy over your non-dominant biceps brachii will be employed to assess local muscle oxygenation. This is a non-invasive procedure whereby a sensor is attached to the biceps brachii and shines an infrared light into the muscle to detect the amount of oxygen the muscle is using.

A more detail verbal explanation will be given if you enrol in the study during your enrolment into the study in order to make an informed consent to this procedure.

Risks and Discomforts

The physical stress associated with this study will be moderate. You may experience moderate muscle soreness after the eccentric exercise session, and you may feel fatigued following eccentric exercise. There may be minor discomfort felt from the electrode placement, but there is no risk of infection. Blood taking risks, including bruising, will be minimal as sterile aseptic techniques will be employed, and ensuring all bleeding stopped before proceeding.

Freedom to withdraw

Your participation in this investigation is voluntary. You may withdraw from any or all parts of the investigation without penalty at any time. You may be withdrawn from the study by the researchers if further participation would be unhealthy for you.

Confidentiality

All questions, answers and results of this study will be treated with absolute confidentiality. Your results will be grouped with those of others for report purposes; however, you will not be individually identifiable.

If you have any further questions or problems connected with your participation in this research, you should contact the chief investigator: Professor Glen Davis on (02) 9351 9466 (working hours) or Mr Sirous Ahmadi, ahmadi1153ir@yahoo.com .

Contact Address: A/Prof Glen Davis/ Mr Sirous Ahmadi, Rehabilitation Research Centre, Faculty of Health Sciences PO Box 170, Lidcombe, 1825. Email: G.Davis@fhs.usyd.edu.au

Essential Information: Any person with concerns or complaints about the conduct of a research study can contact the Manager of Ethics Administration, The University of Sydney on Tel. (02) 9351 4811, Fax (02) 9036 9310

The University of Sydney
Faculty of Health Sciences *Rehabilitation Research Centre*

CONSENT FORM

Muscle oxygenation and muscle blood flow during and after eccentric exercise using near infrared spectroscopy

Investigators: A/Prof Glen Davis, Mr Sirous Ahmadi and Mrs Nasim Foroughi of the Rehabilitation Research Centre, The University of Sydney,

I, _____
[Name]

have read and understood the information for participants on the above named research study and have discussed it.

I am aware of the procedures involved in the study, including any inconvenience, risk, discomfort or side effects and their implications.

I freely chose to participate in this study and understand that I can withdraw at any time without penalty or prejudice.

I also understand that the individual data in this research study is strictly confidential.

I hereby agree to participate in this research study.

Name:
……

Signature:……
…

Date:
……

Essential Information: Any person with concerns or complaints about the conduct of a research study can contact the Manager of Ethics Administration, The University of Sydney on Tel. (02) 9351 4811, Fax (02) 9036 9310, or email gbriody@mail.usyd.edu.au

The University of Sydney
Faculty of Health Sciences

Participant Information Sheet

Muscle oxygenation and muscle blood flow before and after downhill walking using near infrared spectroscopy

You are invited to take part in a research project titled *"***Muscle oxygenation and muscle blood flow before and after downhill walking, using near infrared spectroscopy***"* being conducted by Associate Professor Glen Davis, Dr. Peter Sinclair and Sirous Ahmadi at the Rehabilitation Research Centre within the Faculty of Health Sciences. The project will investigate how we can estimate the quantity and the quality of muscle soreness and/or damage via monitoring muscle blood flow and oxygenation. This research is conducted as a component of the PhD studies of Mr Sirous Ahmadi.

You have been selected for this study because you are aged 18-45, not a competitive sportsperson but otherwise healthy, and without any recent leg muscle injuries. You also must not be on any prescription medications. You will be excluded from participation if you have had a leg muscle injury (of any type) or orthopaedic injuries within the past 12 months, if you perform lower-body strength training more often than once per week, if you have had recent leg muscle biopsy, or if you cannot attend the research laboratory for four consecutive days

Background
During many sport activities and unaccustomed eccentric exercise, the muscles undertake excessive pressures, which may result in delayed onset of muscle soreness. The quantity and quality of exercise-induced delayed onset of muscle soreness has been evaluated both directly and indirectly. However, due to the invasive nature of direct studies, indirect methods to evaluate delayed onset of muscle soreness such as measuring changes in blood biochemistry, a person's perceived muscle soreness, their maximum voluntary strength, muscle electromyography, and other physiological measurements have been preferred to use in human studies. However, the accuracy and reproducibility of these measurements is still unclear.

Oxygen plays a crucial role in energy metabolism and the oxygenation rate changes due to metabolic problems after soreness. The structural changes, increased intramuscular pressure, or increased water content observed in muscle after eccentric exercise may restrict local blood flow and increase the diffusion distance of oxygen. Near infrared spectroscopy is a simple and non-invasive method to monitor oxygen delivery and phosphocreatine recovery in skeletal muscle in healthy and diseased humans.

What do I need to do?
In the current study, we will non-invasively measure your muscle blood flow and oxygenation before and after a session of downhill walking by means of near infrared spectroscopy. Your maximum strength, some blood biochemical measures, thigh

circumference and perceived soreness, will be measured on the first day when you do downhill walking and for the following 4 days in recovery.

We will ask you to attend laboratory testing sessions for approximately 4-5 hours on day 1 and 2 hours the following 4 days. On the first day after pre-tests, you will do 40 mins downhill walking on a treadmill with a gradient up to 25% and speed up to 6.4km/h.

A range of measurements will be assessed before and after walking session on day 1 and every 24hr for 4 consecutive days thereafter. Blood measurements of creatine kinase will be taken via withdrawing approximately 5ml blood from a vein in your arm by a researcher skilled with blood drawing techniques before and after walking session, and at days 3 and 5 thereafter. This is a simple and minimally invasive procedure that will be done via strict aseptic methods by experienced personnel to prevent complications. Blood creatine kinase is collected because it is a biochemical marker of muscle soreness.

We will measure your muscle circumference at the middle of your thigh, while your legs are in a neutral standing position. Soreness when palpating your thigh muscles during flexion and extension of the knee joint, as sometimes occurs with delayed onset of muscle soreness, will be evaluated by a visual analog scale that has 10 point line with "not sore at all" on one side (0) and "very, very sore" on the other side (10). Finally, your maximum voluntary contraction will be measured with your knee joint at 90° whereby 5-second repetitions (3 times) will be performed with 1 min of rest between each contraction. We also will evaluate your muscle oxygenation while you are performing 6 reps of isometric contractions; each contraction lasts 20 seconds with 30, 50 and 80% of maximum voluntary contraction.

A probe of near infrared spectroscopy, fastened over your dominant leg will be employed to assess local muscle oxygenation. This is a non-invasive procedure whereby a sensor is attached to the quadriceps muscle and shines an infrared light into the muscle to detect the amount of oxygen the muscle is using.

A more detail verbal explanation will be given if you enrol in the study during your enrolment into the study in order to make an informed consent to this procedure.

Risks and Discomforts

The physical stress associated with this study will be moderate. You may experience moderate muscle soreness and/or fatigue after the walking session. There may be minor discomfort felt from the probe placement, but there is no risk of infection. Blood taking risks, including bruising, will be minimal as sterile aseptic techniques will be employed, and ensuring all bleeding stopped before proceeding.

Freedom to withdraw
Your participation in this investigation is voluntary. You may withdraw from any or all parts of the investigation without penalty at any time. You may be withdrawn from the study by the researchers if further participation would be unhealthy for you.

Confidentiality
All questions, answers and results of this study will be treated with absolute confidentiality. Your results will be grouped with those of others for report purposes; however, you will not be individually identifiable.

If you have any further questions or problems connected with your participation in this research, you should contact the chief investigator: Professor Glen Davis on (02) 9351 9466 (working hours) or Mr Sirous Ahmadi, ahmadi1153ir@yahoo.com.

Contact Address: A/Prof Glen Davis/ Mr Sirous Ahmadi, Rehabilitation Research Centre, Faculty of Health Sciences PO Box 170, Lidcombe, 1825. Email: G.Davis@fhs.usyd.edu.au

Essential Information: Any person with concerns or complaints about the conduct of a research study can contact the Manager of Ethics Administration, The University of Sydney on Tel. (02) 9351 4811, Fax (02) 9036 9310

The University of Sydney
Faculty of Health Sciences *Rehabilitation Research Centre*

CONSENT FORM

Muscle oxygenation and muscle blood flow before and after downhill walking, using near infrared spectroscopy

Investigators: A/Prof Glen Davis, Dr. Peter Sinclair and Mr Sirous Ahmadi of the Rehabilitation Research Centre, The University of Sydney,

I, _____
[Name]

have read and understood the information for participants on the above named research study and have discussed it.

I am aware of the procedures involved in the study, including any inconvenience, risk, discomfort or side effects and their implications.

I freely chose to participate in this study and understand that I can withdraw at any time without penalty or prejudice.

I also understand that the individual data in this research study is strictly confidential.

I hereby agree to participate in this research study.

Name:
……

Signature:……
…

Date:
……

Essential Information: Any person with concerns or complaints about the conduct of a research study can contact the Manager of Ethics Administration, The University of Sydney on Tel. (02) 9351 4811, Fax (02) 9036 9310, or email gbriody@mail.usyd.edu.au

The University of Sydney
Faculty of Health Sciences

Participant Information Sheet

Muscle oxygenation and muscle blood flow during and after concentric exercise using near infrared spectroscopy

You are invited to take part in a research project titled *"Muscle oxygenation and muscle blood flow during and after concentric exercise using near infrared spectroscopy"* being conducted by Associate Professor Glen Davis, Dr. Peter Sinclair and Mr. Sirous Ahmadi at the Rehabilitation Research Centre within the Faculty of Health Sciences. The project will monitor muscle oxygenation and blood flow before, during and after a session of concentric contractions. This research is conducted as a component of the PhD studies of Mr Sirous Ahmadi.

You have been selected for this study because you are male, aged 18-45, not a competitive sportsperson but otherwise healthy, and without any recent arm muscle injuries. You also must not be on any prescription medications. You will be excluded from participation if you have had an arm muscle injury (of any type) or orthopaedic injuries within the past 12 months, if you perform upper-body strength training more often than once per week, if you have had recent arm muscle biopsy, or if you can not attend the research laboratory for three consecutive days

Background
Oxygen plays a crucial role in energy metabolism, and the oxygenation rate changes due to metabolic problems. Increased energy expenditures usually require rapid adjustments in blood flow and muscle oxygenation that affect the entire cardiovascular system. During exercise, the vascular portion of active muscles is considerably increased by the dilation of local arterioles. Furthermore, increase in acidity, temperature, or concentration of carbon dioxide in exercising muscles causes an increase in oxygen release from oxygenated haemoglobin. Therefore, during exercise there is a decline in muscle oxygen saturation and it is expected that oxygen saturation recovers within a short period after exercise. However, our previous unpublished studies have shown some long lasting changes in muscle oxygenation during rest and exercise after heavy eccentric exercises.

Near infrared spectroscopy (NIRS), is a simple and non-invasive method to monitor oxygen delivery and consumption in skeletal muscle in healthy and diseased humans. NIRS has been employed during both static and deoxygenated form, whereas at 850 nm they occur in the oxygenated state. in this study we use NIRS to monitor muscle oxygenation in your arm muscles.

What do I need to do?
In the current study, we will non-invasively measure your muscle blood flow and oxygenation before, during and after a session of concentric exercise by means of NIRS.

Your maximum strength, some blood biochemical measures, your elbow joint range of motion, arm circumference and perceived soreness will be measured on the first day when you do strenuous arm exercise and for the following 2 days in recovery.

We will ask you to attend laboratory testing sessions for 3 hours each day over a period of 3 days. On the first day, you will perform 2 bouts of 35 maximal voluntary concentric contractions with the elbow flexors of your non-dominant arm placed in an isokinetic dynamometer moving at a slow speed (60 deg•s^{-1}).

A range of measurements will be assessed before and after concentric exercise on day 1, and every 24hr for two consecutive days thereafter. Blood measurements of creatine kinase and myoglobin will be taken via withdrawing approximately 5ml blood from a vein in your arm by a researcher skilled with blood drawing techniques before and after the concentric exercise session, and on days 2 and 3 thereafter. This is a simple and minimally invasive procedure that will be done via stringent aseptic methods by experienced personnel to prevent complications. Blood creatine kinase and myoglobin is collected because it is a biochemical marker of muscle soreness. Furthermore, near infrared spectroscopy (described below) will be done during the concentric exercise session.

Your relaxed and flexed elbow joint angles will be assessed three times using an angle-measuring device (called a “goniometer”), and the difference between the two angles will be used as your joint range of motion around the elbow joint. We will measure your arm circumference at 4, 6, 8, and 10 cm above the elbow joint, while allowing your arm to hang down by the side. Soreness when palpating the upper arm during flexion and extension of the elbow joint, as sometimes occurs with delayed onset of muscle soreness, will be evaluated by a visual analog scale that has 7 points with "not sore at all" on one side (0) and "very, very sore" on the other side (7). Finally, your maximum voluntary contraction will be measured with your elbow joint at 90° whereby 5-second repetitions (3 times) will be performed with 1 min of rest between each contraction. We also will evaluate your muscle oxygenation while you are performing 3 bouts of isometric contractions; each contraction lasts 20 seconds with 80% of maximum voluntary contraction.

Dual-channel near infrared spectroscopy over your non-dominant biceps brachii will be employed to assess local muscle oxygenation. This is a non-invasive procedure whereby a sensor is attached to the biceps brachii and shines an infrared light into the muscle to detect the amount of oxygen the muscle is using.

A more detail verbal explanation will be given if you enrol in the study during your enrolment into the study in order to make an informed consent to this procedure.

Risks and Discomforts
The physical stress associated with this study will be moderate. You may experience moderate muscle fatigue after the concentric exercise session. There may be minor discomfort felt from the NIRS probe placement, but there is no risk of infection. Blood taking risks, including bruising, will be minimal as sterile aseptic techniques will be employed, and ensuring all bleeding stopped before proceeding.

Freedom to withdraw
Your participation in this investigation is voluntary. You may withdraw from any or all parts of the investigation without penalty at any time. You may be withdrawn from the study by the researchers if further participation would be unhealthy for you.

Confidentiality
All questions, answers and results of this study will be treated with absolute confidentiality. Your results will be grouped with those of others for report purposes; however, you will not be individually identifiable.

If you have any further questions or problems connected with your participation in this research, you should contact the chief investigator: Professor Glen Davis on (02) 9351 9466 (working hours) or Mr Sirous Ahmadi, sahm8027@mail.usyd.edu.au

Contact Address: A/Prof Glen Davis/ Mr Sirous Ahmadi, Rehabilitation Research Centre, Faculty of Health Sciences PO Box 170, Lidcombe, 1825. Email: G.Davis@fhs.usyd.edu.au

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The University of Sydney
Faculty of Health Sciences *Rehabilitation Research Centre*

CONSENT FORM

Muscle oxygenation and muscle blood flow during and after concentric exercise using near infrared spectroscopy

Investigators: A/Prof Glen Davis, Dr. Peter Sinclair and Mr Sirous Ahmadi of the Rehabilitation Research Centre, The University of Sydney,

I, _____
[Name]

have read and understood the information for participants on the above named research study and have discussed it.

I am aware of the procedures involved in the study, including any inconvenience, risk, discomfort or side effects and their implications.

I freely chose to participate in this study and understand that I can withdraw at any time without penalty or prejudice.

I also understand that the individual data in this research study is strictly confidential.

I hereby agree to participate in this research study.

Name:
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